Thermal adaptation in the lizard *Cordylus oelofseni*: physiological and behavioural responses to temperature variation

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Declaration

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Abstract

As ectotherms, lizards are particularly vulnerable to changes in the thermal landscape and face extinction risk if they lack the capacity to rapidly adapt or behaviourally mitigate increasingly altered thermal environments. Theoretical models that predict lizards' response to climate change often fail to take into account the thermal characteristics of the microenvironment, the ability of lizards to behaviourally buffer climate variation in the habitat and the plastic nature of both behaviour and physiology over ecologically relevant time-scales. Here, I address this major knowledge gap using two separate research chapters in an experimental physiology approach. In Chapter 1, I investigated the temperature-dependence and plasticity of resting metabolic rate, water-loss rate and preferred body temperature of *Cordylus oelofseni* at several temporal scales (within and between seasons) and incorporated field observations to acquire a better understanding of this species' adaptive potential to buffer thermal changes in the habitat. *Cordylus oelofseni* showed plasticity of both behaviour and physiology in response to thermal acclimation, but relied on distinct strategies depending on the time-scale investigated. These results highlighted the complexity of underlying mechanisms used by these organisms to buffer temperature variation. In Chapter 2, I used an experimental approach to examine the energetic costs of thermoregulation in *C. oelofseni* and test the cost-benefit model of thermoregulation. This model's primary prediction states that lizards should thermoregulate carefully only when the associated costs are low. Using four enclosures that simulated different thermal qualities (temporal and spatial distributions of operative temperatures) in the habitat, I found limited support for the cost-benefit model. Lizards in the low-quality heterogeneous enclosures invested the same energetic effort and thermoregulated with similar overall accuracy as lizards in the high-quality heterogeneous enclosure. The costs incurred were not necessarily energetic, but reflected missed opportunities (e.g. less time to forage), something that, along with important interaction effects with body mass, deserves further attention when testing this model. Together, these results illustrate the importance of incorporating ecological reality at various time and spatial scales in order to make relevant predictions regarding the fate of lizards with projected climate change.

Opsomming

As ektotermiese diere, is akkedisse veral sensitief vir veranderinge in die termiese landskap en staar uitsterwingsrisiko in die gesig as hulle nie die vermoë het om vinnig aan te pas of gedragsveranderinge te maak in omgewings wat toenemend verwarm nie. Teoretiese modelle wat akkedisse se reaksie op klimaatsverandering voorspel, neem dikwels nie die termiese eienskappe van die mikro-omgewing, die vermoë van akkedisse om met gedragsveranderinge klimaat variasie in die habitat te buffer en die plastieke aard van beide gedrag en fisiologie oor ekologies relevante tydskale in ag nie. Hier bespreek ek hierdie groot kennisgaping met behulp van twee afsonderlike navorsingshoofstukke in 'n eksperimentele fisiologie benadering. In Hoofstuk 1 het ek ondersoek ingestel na die temperatuur-afhanklikheid en plastisiteit van rustende metaboliese tempo, waterverlies tempo en voorkeur liggaamstemperatuur van *Cordylus oelofseni* by verskeie tydskale (binne en tussen seisoene) en inkorporeer veld waarnemings om 'n beter begrip te verkry van hierdie spesie se aanpasbare potensiaal om termiese veranderinge in die habitat te buffer. *Cordylus oelofseni* het plastisiteit van beide gedrag en fisiologie in reaksie op hitte-akklimatisering getoon, maar staatgemaak op verskillende strategieë, afhangende van die tyd-skaal wat ondersoek is. Hierdie resultate beklemtoon die kompleksiteit van die onderliggende meganismes wat gebruik word deur hierdie organisme om temperatuur verandering te buffer. In Hoofstuk 2 het ek 'n eksperimentele benadering gebruik om die energiekoste van termoregulering in *C. oelofseni* te ondersoek en die kostevoordeel model van termoregulering te toets. Hierdie model se primêre voorspelling verklaar dat akkedisse slegs versigtig moet termoreguleer wanneer die gepaardgaande koste laag is. Deur gebruik te maak van vier afskortings wat verskillende termiese eienskappe gesimuleer het (tyd en ruimtelike verspreiding van operatiewe temperature) in die habitat, het ek beperkte ondersteuning gevind vir die koste-voordeel model. Akkedisse in die lae-gehalte heterogene afskortings het dieselfde energieke moeite belê en getermoreguleer met soortgelyke algehele akkuraatheid as akkedisse in die hoë-gehalte heterogene kamp. Die kostes wat aangegaan is, is nie noodwendig energiek nie, maar weerspieël geleenthede wat gemis is (bv. minder tyd om kos te soek), iets wat, saam met belangrike interaksie effekte met liggaamsmassa, verdere aandag verdien wanneer hierdie model getoets word. Tesame illustreer hierdie resultate die belangrikheid van die integrasie van ekologiese werklikheid op verskillende tyd en ruimtelike skale, om relevante voorspellings oor die lot van akkedisse met geprojekteerde klimaatsverandering te kan maak.

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RMR	resting metabolic rate
WLR	water-loss rate
T_{sel}	preferred body temperature
CT_{max}	critical thermal maximum
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}H_2O$	rate of water-loss
T_e	operative temperature
T_b	body temperature
SVL	snout-vent length
M_b	body mass
TT	test temperature
ACC	acclimation
H`	Shannon-Wiener's measure of diversity
$E_{1/D}$	Simpson's evenness measure
d	Berger-Parker's dominance index
CB model	cost-benefit model of thermoregulation
IR	infra-red
$\overline{d}_e$	thermal habitat quality
$\overline{d}_b$	accuracy of thermoregulation
E	thermoregulatory efficiency
AMR	active metabolic rate
LQ homog	low-quality homogeneous
LQ heterog	low-quality heterogeneous
HQ homog	high-quality homogeneous
HQ heterog	high-quality heterogeneous

General introduction

With increasing concern over global extinction rates as a result of climate change, a growing number of studies focus on the response of ectotherms (from insects to reptiles) to changes in their thermal environment (Araújo *et al*. 2006; Deutsch *et al*. 2008; Hughes 2008; Huey *et al*. 2010). Ectotherms provide excellent model systems to investigate the underlying mechanisms of species physiological and behavioural responses to variation in the thermal landscape. Ectotherms have low metabolic heat production, generating most body heat from their physical surroundings (through solar radiation, convection and conduction) and since temperature drives all major biological and physiological processes, they are highly vulnerable to changes in their abiotic environment (Adolph & Porter 1993; Angilletta *et al*. 2002; Martin & Huey 2008; Angilletta 2009). In addition, physiological and morphological modifications such as increased blood flow (Baker *et al*. 1972) and changes in melanin content of the skin/elytra (De Jong *et al*. 1996; Clusella-Trullas *et al*. 2009) are fairly minor contributions to dealing with changes in the thermal quality of the environment. In fact, a large proportion of ectotherms predominantly rely on behavioural adjustments (e.g. shuttling in lizards) to buffer the thermal heterogeneity (spatial and temporal) encountered in their habitats (Huey 1974; Kührt *et al*. 2005; Golovanov 2006).

Apart from behavioural modifications, evolutionary changes can also take place, sometimes occurring rapidly due to the short life-span of some taxa (e.g. insects), allowing them to adapt to the changing environment across generations (Hoffmann & Sgrò 2011). Rapid evolutionary changes are likely less relevant for larger ectotherms, such as reptiles which typically have longer life-spans. However, short- or long-term changes in physiology and behaviour can also be achieved through phenotypic plasticity, maximizing fitness (Angilletta 2009). Finally, if ectotherms cannot respond rapidly enough to changes in their thermal environment, they may face extinction. This last case scenario is more pervasive than previously thought, especially for lizards. Sinervo *et al*. (2010) modelled the extinction risk of lizards globally using historic and current data on extinction rates of lizard species and projected a 20% decline in lizard populations globally by 2080 as a result of climate change. Similarly, there is a global concern for lizard persistence in tropical forests as species tend to have lower heat tolerance and adaptive capacity (Huey *et al*. 2009; Kearney *et al*. 2009; Gunderson & Leal 2012, but see Logan *et al*. 2013). As increasing temperature elevates ectotherm metabolic rates, and thus, energy requirements, energetic constraints can also limit population densities (Buckley 2008; Dillon *et al*. 2010), and thus, can impact ecological systems via cascading trophic effects. Given the current fast rate of climate change, plastic responses will be particularly important for species that have reduced adaptation potential (long generation time, small founder population size, limited genetic variation and low fecundity) and for which dispersion is prevented, due to, for example, habitat fragmentation or thermal constrains originating from reduced habitat quality. It is clear that there is a need to study the capacity of ectotherms to cope with changing thermal landscapes as the inability to evolve rapidly will have obvious negative fitness consequences. Perhaps more importantly is an improved understanding of the

microenvironment which they inhabit and the suite of strategies they can employ such as physiological and behavioural adjustments, and their relative contributions towards buffering climate variation. Quantifying the costs, benefits and limits of their adaptive (including plastic) capacity will determine their persistence in their current distribution ranges and highlight the degree of evolutionary change needed to counteract future climate scenarios (Somero 2010; Chevin *et al*. 2010; Reed *et al*. 2011).

Therefore, in order to examine the contributions of physiological and behavioural mechanisms, I chose a small lizard, *Cordylus oelofseni* (Sauria: Cordylidae) as a model organism. *C. oelofseni* is a melanistic, viviparous and highly endemic species restricted to mountain tops in the Hottentots Holland Mountains, Western Cape, South Africa. Its life history traits and restricted distribution makes this species likely vulnerable to climatic change. In Chapter 1, I explore the direction and magnitude of plastic responses of thermoregulatory behaviour, metabolism and water balance of this species and determine which strategies take place at different temporal scales (weather fronts and seasons) using ecologically-relevant time frames and acclimation regimes. In Chapter 2, I test the primary prediction of an optimality model of thermoregulation (the cost-benefit model of thermoregulation, Huey & Slatkin 1976) using an energy budget approach. By testing such a model, improved predictions on physiological and behavioural responses of ectotherms when facing altered thermal quality in their environments can be made.

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Chapter 1

Ecological reality highlights different contributions of behavioural and physiological compensation across time-scales in a lizard

1.1 Introduction

Concurrent to forecasting population responses to climate change, there is a need to understand the potential for vulnerable species to buffer climate variation. In lizards, while much emphasis has been given to the means of performance curve parameters (typically, critical thermal limits and temperature dependence of running speed or metabolism, Marsh & Bennett 1986; White *et al*. 2006; Angilletta *et al*. 2010), less attention has been given to the plasticity of physiological and behavioural traits (Clusella-Trullas & Chown *in press*), especially at an ecologically-relevant scale (Gvoždík & Castilla 2001). In particular, intra-individual shifts in thermoregulatory behaviour, metabolism and water-loss may compensate thermal variation but the temporal scales at which these shifts may incur (extreme events, weather fronts or seasons) are also largely unknown.

Lizards depend on their micro-environment and use behavioural adjustments (body orientation, posture and shuttling) to maintain body temperature within a range optimal for performance. Traits of performance described as a function of body temperature typically have an asymmetrical shape which generally peaks at the preferred body temperature (targeted temperature selected in a thermal gradient devoid of biotic factors, T_{sel}) but rapidly drops at temperatures higher than T_{sel}. At T_{sel}, most physiological and biological processes are optimised (enzyme activity, energy assimilation, sprinting, Licht 1964; Hertz *et al*. 1983, but see Angilletta *et al*. 2002) and likely enhance individual fitness (Cowles & Bogert 1944; Huey & Bennett 1987; Angilletta *et al*. 2006). However, by targeting T_{sel}, thermoregulatory behaviours can rapidly become energetically expensive, especially during periods of poor thermal quality or in low quality habitats (Lee 1980; Anderson & Karasov 1981, see the cost-benefit model of thermoregulation by Huey & Slatkin 1976). Plastic responses in the form of acclimation or acclimatization of T_{sel} may compensate for climate variation and favour performance while minimizing costs and optimizing activity periods in sub-optimal conditions (Hadamová & Gvoždík 2011).

Seasonal shifts in T_{sel} have been reported (acclimatization) (reviewed in Clusella-Trullas & Chown *in press*) but responses to shorter exposures (e.g. acclimation, Angilletta 2009) have provided mixed effects (e.g. Wheeler 1986; Kaufmann & Bennett 1989; Clusella-Trullas & Chown *in press*). In general, T_{sel} is thought to be largely conserved among lizards, possibly due to the "Bogert effect" (Huey *et al*. 2003), whereby evolution of physiological traits are constrained as a result of behavioural adjustments, minimising selection (Losos *et al*. 2004). Recently, Gvoždík (2012) suggested three likely reasons for which the plasticity of T_{sel}, although existent in some species, has largely been overlooked: 1. acclimation exposures typically lack biological reality (e.g. no diel fluctuations as in natural regimes), 2. the magnitude of acclimation responses is typically small (but nonetheless significant) and 3. acute (reactive) thermoregulatory adjustment may be considered more important than plastic responses. In addition, a shift in T_{sel} may require high energetic costs and other compensatory responses may instead take place. For example, under a shift to a warmer thermal

regime, a beneficial plastic response should involve a positive shift of T_{sel} to maintain performance under the new environmental conditions. This response may however have detrimental consequences as the margin between T_{sel} and the critical thermal maximum (CT_{max}) is typically small, and T_{sel} is generally maintained below T_{opt} (Martin & Huey 2008). This scenario would also mean that optimal performances (e.g. running speed, metabolic capacity) should encompass or shift closer to the new T_{sel} although most biochemical processes are also rapidly bound by high temperatures. Instead, T_{sel} may not shift under warm conditions but the thermal sensitivity of some physiological traits such as maintenance metabolism (resting metabolic rate, RMR) may compensate for temperature changes by lowering energetic costs (Tsuji 1988a). Indeed, understanding beneficial (adaptive) acclimation responses requires examination of several traits concomitantly, not just one (Kingsolver & Huey 1998; Woods & Harrison 2001; Glanville & Seebacher 2006). In addition, compensation of physiological traits is costly in terms of time and energy required to synthesize proteins and other cellular processes (e.g. rates of transcription; Somero 1978; Angilletta *et al*. 2006; Rogers *et al*. 2007; Burton *et al*. 2011) which may limit the magnitude of plasticity of these traits. Overall, little is still known about which behavioural and/or physiological acclimatory responses or combination thereof, should be expected under different climate regimes.

In thermally variable environments, lizard species that hibernate typically reduce their RMR, lowering energetic costs when resources are scarce (Congdon *et al*. 1979) whereas species that stay active in winter generally have increased RMR (Tsuji 1988a). The latter is in line with the thermal compensation or metabolic cold adaptation hypothesis (MCA), whereby individuals exposed to a cold temperature regime maintain a higher RMR than warm exposed individuals when tested at a common intermediate temperature (McNab 2002). A high RMR enables the maintenance of the metabolic machinery during climatic changes and allows lizards to perform activities such as thermoregulatory behaviour and foraging, and thus, is likely dependent on the availability of resources (Clarke 1993, 2003). The occurrence of MCA in lizards is pervasive in the literature (see Tsuji 1988a for review, Hare *et al*. 2010) while support for metabolic compensation in summer, whereby RMR is lowered to limit energetic costs, is limited (Tsuji 1988a; Christian & Green 1994). In this case, a lowering of RMR in summer may also decrease water-loss when water resources are scarce (Claussen 1967; Case 1976). Alternatively, if water is available, evaporative cooling may be a complementary strategy to maintain optimal temperatures in summer.

This study aims to investigate the direction and magnitude of behavioural and physiological plasticity and determine which strategies take place at different temporal scales (short-term, such as during transient weather fronts, and between summer and winter seasons). For this, I investigate thermal acclimation responses of preferred body temperature, resting metabolic rate and water-loss rate of *Cordylus oelofseni* (Mouton & van Wyk 1990), a diurnal, temperate viviparous lizard that inhabits rock outcrops and maintains activity throughout winter. Cordylid lizards are good thermoregulators (Clusella-Trullas *et al*. 2009) and have relatively long life spans (~10 - 20 years; Fogel 2003) and thus,

should experience substantial seasonal and inter-annual variation within generations, making this species an excellent model to investigate the plasticity of these traits and potential compensatory roles thereof. In addition, in order to increase ecological relevance in the interpretation of plastic responses, I define acclimation treatments from field operative temperature distributions, examine lizard thermal habitat quality and behavioural thermoregulation in the field and assess insect diversity and abundance as a measure of resources available in summer and winter.

1.2 Materials and Methods

1.2.1 Lizard collection and maintenance

Lizards were collected (21 males, 21 females) from Landdroskop (34°02' S, 19°00' E, altitude 1080 m asl) in the Hottentots Holland Mountains, Western Cape Province, South Africa, in winter (1 - 2 August 2011) and summer (12 - 15 January 2012). Lizards were individually marked with non-toxic paint and transported to the laboratory to begin acclimation treatments within 48 hrs. Individuals were maintained in dark cloth bags within a cooler box during transport to avoid stress and heat. Ticks and mites were found on summer collected lizards and removed using a toothpick and 99% ethanol prior to experimental procedures.

After determining M_b (Analytical balance, ± 0.0001 g, AX504, Mettler Toledo International Inc.), sex and SVL, lizards were housed in terrariums (plastic containers with mesh tops, 9.5 L, max. four individuals per container) with stone/sand substrate and artificial refuges and placed within a temperature-controlled incubator (Sanyo Cooled Incubator, MIR - 254, Sanyo Electric Co., Ltd., Japan). Water was supplied *ad libitum* while food (grey crickets *Acheta domestica* dusted in a vitamin and mineral supplement and superworms *Zophobas morio*) was given once a week during winter and twice a week during summer. Ultra-violet light was provided daily and the containers were rotated on a weekly basis to ensure that all lizards were equally exposed to acclimation treatments and UV-B radiation. In order to monitor lizard condition, individual body mass (M_b) was measured weekly.

1.2.2 Seasonal thermal regimes and acclimations

At the study site, 26 calibrated copper models (hollow copper models of the same size, shape (including legs and tail), and reflectance as *C. oelofseni*) randomly placed in a variety of microhabitats (sun, shade, and crevice; see Clusella-Trullas *et al*. 2009 for details) and connected to a datalogger (CR1000 and 32 channel multiplexer, Campbell Scientific, Utah, USA) were placed to measure and record the operative temperatures (T_e) of *C. oelofseni* in its habitat at an hourly rate. The models are kept at the same microsites as part of a long-term monitoring project assessing T_e variation at the study location. Acclimation treatments in the laboratory were based on T_e data from summer (January - March) and winter (June - August) of 2005, 2011 and 2012 and mimicked diel cycles experienced by lizards for each season (Table 1.1).

Lizards were first exposed for one week to the 'Average' acclimation (ACC) temperature for that season and then split into three ACC treatments ('Cold', 'Average' and 'Warm', Table 1.1) with 14 lizards per treatment (seven males, seven females). Lizards were maintained at these acclimation temperatures for two weeks before I started the first respirometry trial and kept in the same conditions until T_{sel} trials were completed (*ca*. one month). Dataloggers (Maxim Hygrochron iButtons, DS1923, Sunnyvale, California, USA) were used to record temperatures every 10 min in the laboratory acclimations and experimental set-ups.

Table 1.1. Summary of operative temperatures (T_e) from 26 lizard copper models and laboratory acclimation treatments in summer and winter. All temperatures are given for day/night. For T_e, 'Average', 'Min' and 'Max' represent mean, 25th and 75th percentiles of T_e data, respectively. For acclimations, 'Cold', 'Average' and 'Warm' are mean temperatures recorded in the incubators during temperature treatments. The photoperiod cycle was 10:14 and 14:10 (light:dark h) for winter and summer, respectively.

Season	Operative temperatures - T_e (°C)	Acclimation treatments (°C)
Winter	Min 6.4 / 4.3	Cold 10.3 ± 0.6 / 7.5 ± 0.5
	Average 12.1 ± 7.2 / 7.0 ± 3.6	Average 14.4 ± 0.8 / 7.1 ± 0.7
	Max 16.6 / 9.2	Warm 19.6 ± 1.3 / 7.4 ± 0.7
Summer	Min 15.8 / 13.3	Cold 19.9 ± 1.2 / 15.4 ± 1.0
	Average 22.6 ± 8.5 / 15.7 ± 3.5	Average 24.2 ± 0.6 / 15.1 ± 1.6
	Max 28.1 / 18.1	Warm 29.1 ± 0.9 / 15.0 ± 0.7

1.2.3 Respirometry

Resting metabolic rate (RMR) and water-loss rate (WLR) were determined using a calibrated LI-COR infra-red CO_2/H_2O analyser (Li-7000, LI-COR Inc., Lincoln, Nebraska, USA) set in gas flow configuration and plumbed in differential mode (Lighton 2008) (Fig. 1.1). The respirometry gas was

supplied by a k-cylinder (21% O_2) which flowed through two scrubber columns, one filled with silica gel:drierite (50:50) for scrubbing water and the other with soda lime for scrubbing CO_2. Thereafter the gas flowed through a mass flow control valve (MF) (Sierra Instruments, Side-Track Model 840, Monterey, California, USA) controlled by a mass flow control unit (Sable Systems, Intelligent Mass Flow Control Unit, MFC-2, Las Vegas, Nevada, USA) at a rate of 200 ml/min. The air then flowed into optical cell A of the LI-COR as reference gas, out of the optical cell A of the LI-COR and into the cuvette (180 ml) containing one lizard. The gas then flowed out of the cuvette and back into the second optical cell B of the IRGA for differential CO_2 recording. A baseline recording was done at the beginning and at the end of each respirometry run. A glass respirometry cuvette was darkened to increase quiescence of lizards and kept in an incubator (Sanyo Cooled Incubator, MIR-153, Sanyo Electric Co., Ltd., Japan) at one of three test temperatures (TT): 10, 20 and 30°C (verified using a thermocouple and recorder TC 1000, Sable Systems, Las Vegas, Nevada, USA) and maintained within 1°C of the target temperature. Each individual was tested at the three TT in a randomised order, resulting in a total of 126 trials. Each recording lasted for a minimum of 40 min at the lower TT (10°C) and a minimum of 30 min at the highest TT (30°C). Periods of resting RMR and WLR were identified during pilot trials as minimum stable periods in the gas exchange trace which matched periods of quiescence. Lizards were fasted for 48 hrs and were given 15 min to habituate to the cuvette and equilibrate to the TT before respirometry trials. Each lizard was weighed before and after each trial.

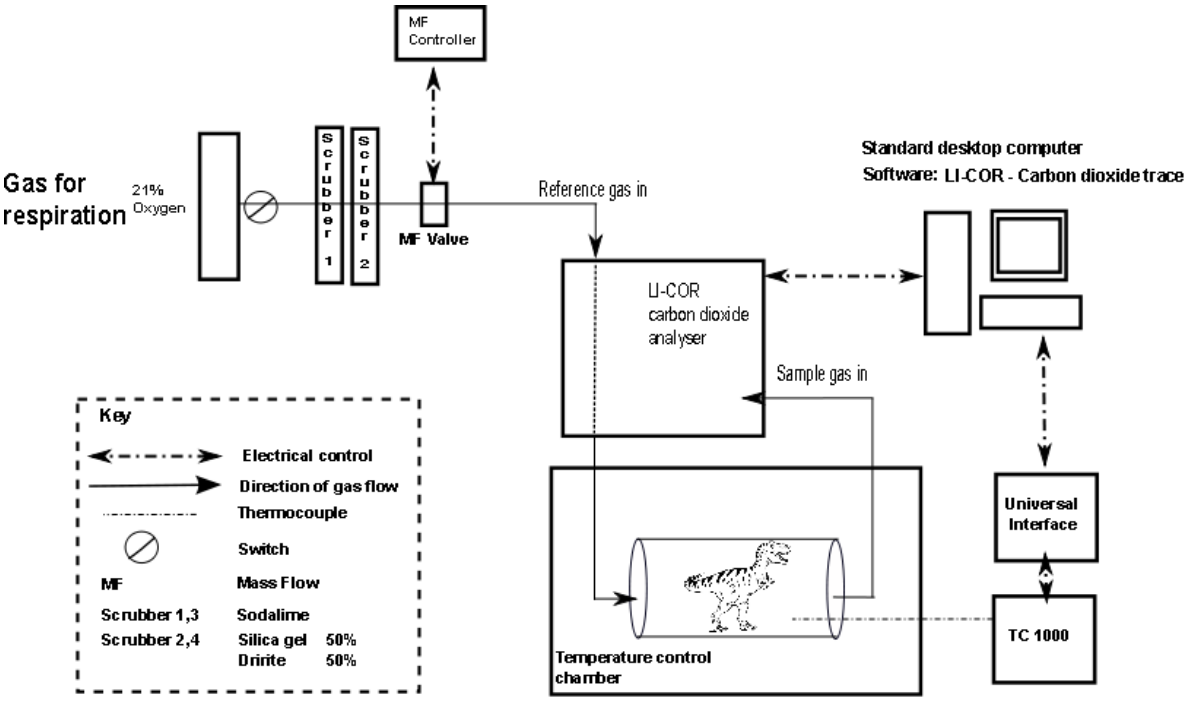

Figure 1.1. Respirometry setup used to measure resting metabolic rate (RMR) and water-loss rate (WLR).

1.2.4 Preferred body temperature

Lizards were given a minimum of one week in their respective acclimation treatments before T_{sel} trials resumed. A temperature gradient (12-55°C, verified using calibrated copper models) was created by placing the gradient in a temperature controlled room (set at 12°C) for the cold end and infra-red lights at the warm end (175 W, General Electric Company, Johannesburg, South Africa). The infra-red lights were suspended *ca*. 30 cm from the ground at each end of five 180 x 30 cm plywood rectangles (with stones as substrate). Thin thermocouples (Type T, 36SWG, Omega Engineering, Inc., Stamford, Connecticut, USA) were inserted into the lizard cloaca and secured with elastic tape (Elastoplast, Durban, KwaZulu Natal, South Africa) and small cable-ties around the tail. The thermocouples were connected to a suspended thicker thermocouple wire (Type T, 24SWG) to allow free movement of the lizard without constraint. Temperatures from five lizards were logged simultaneously at 5 min intervals using a CR1000 datalogger. Water was provided *ad libitum* in plastic petri dishes, placed *ca*. 10 cm from the cold end of the box. Lizard body temperature (T_b) was recorded while roaming free in the gradient from 9:30 to 16:00 and their M_b recorded before and after T_{sel} trials.

1.2.5 Field lizard activity and prey availability

To assess the abundance and activity patterns of *C. oelofseni* in the field, I walked two 100 m transects daily at 08:00, 10:00, 12:00, 15:00 and 18:00 for four days during summer (January) and winter (August). The number of lizards within 10 m on either side of the transects were counted and activities recorded (e.g. basking, walking, mating, running). Prey availability was determined for both seasons by use of pitfall traps (n = 21, 200 ml plastic containers filled with 75% EtOH (50 ml) and dishwashing liquid (5 ml); Zytynska *et al*. 2011). Traps were opened at 08:00 and closed at 18:00 for five days to catch insects during lizard activity periods. Additionally, net sweeping through the adjacent vegetation was done for 15 min, at 09:00, 13:00 and 17:00 for four consecutive days, in order to catch insects that do not fall into pitfall traps but form part of these lizards' diet (Clusella-Trullas & Botes 2007).

1.2.6 Statistical analyses

For the summer season, only non-gravid females and male lizards were included in the analyses. For each season, RMR ($\dot{V}CO_2$ ml/h) was log-transformed and analysed using a general mixed effects model (nlme package, Pinheiro *et al*. 2013) with acclimation, test temperature, body mass and sex as fixed factors and lizard (identity) as a random factor. WLR ($\dot{V}H_2O$ mg/h) was similarly analysed. Differences in RMR and WLR between winter and summer lizards were assessed using data at TT of 30°C as it reflects the closest temperature to T_{sel}. The residuals from the regression of RMR and WLR against average M_b were used as the dependent variables and analysed using a generalised linear model (GLZ), with season, ACC and sex as predictors. To further explore seasonal effects, I compared data from the 20°C ACC only (common ACC to both seasons) using a general mixed effects model

(RMR or WLR as the dependent variable and season, test temperature, body mass and sex as fixed factors and lizard (identity) as the random factor).

The central 50% of T_{sel} data (for summer and winter separately) were used to determine the mean, minimum (25^{th} quartile), maximum (75^{th} quartile), and range of T_{sel} selected, since extreme values were considered to be lizard exploratory behaviour (Hertz *et al*. 1993). GLZ models (with Gaussian family and identity link function) were used to analyse T_{sel} data. Mean, minimum, maximum and range of T_{sel} selected were treated as dependent variables and season, acclimation and sex treated as categorical variables. Body mass (M_b) was treated as a continuous variable in all analyses.

Thermal habitat quality ($\overline{d}_e$) was calculated as the deviance of T_e from T_{sel} ($\overline{d}_e = T_{sel} - T_e$; Hertz *et al*. 1993) using T_e data taken from three months of summer (January - March 2012) and winter (June - August 2011). Smaller values of ($\overline{d}_e$) reflect better habitat quality and summer and winter ($\overline{d}_e$) were compared using two-sample t tests. For comparisons of prey abundance and diversity between seasons, arthropods were identified to family level. Species were assigned species numbers for identification and use in the indices below. Three indices were used to summarise the data: Shannon-Wiener's measure of diversity (H`), Simpson's evenness measure ($E_{1/D}$) and Berger-Parker's dominance index (d). Absolute abundance and species richness are also reported.

All statistical analyses were conducted in R (v. 2.15.0, R Development Core Team). A significant level of $\alpha < 0.05$ was set for all statistical tests. All results presented in Tables are minimal adequate models based on Akaike Information Criteria and general mixed effects models were compared using maximum likelihood (Crawley 2007). Outliers for the mixed effects models were identified from plots of standardised residuals vs. fitted values and from Cook's distance criteria for the GLZ models (e.g. Packard & Boardman 2009, maximum number of outliers for T_{sel} = three and WLR = four). Means are reported ± s.d. unless stated otherwise.

1.3 Results

1.3.1 Metabolism and water-loss

In summer, both RMR and WLR were significantly positively related to TT and M_b but acclimation did not have an effect on these response variables (Table 1.2). In winter, both RMR and WLR increased significantly with TT and M_b (Table 1.2). For RMR, ACC and ACC*TT effects were significant. Warmer acclimation resulted in higher RMR and the interaction indicated that individuals acclimated at the warmest ACC (20°C) had a higher RMR than individuals acclimated to 10 and 15°C at the lowest TT (10°C) whereas individuals acclimated at (10°C) had higher RMR than individuals acclimated to 15 or 20°C at the warmest TT (30°C) (Table 1.2, Fig. 1.2).

Table 1.2. General mixed effect model for the effect of test temperature (TT), acclimation (ACC), body mass (M_b) and sex on resting metabolic rate (RMR; $\log\dot{V}CO_2$ (ml/h)) and water-loss rates (WLR; $\dot{V}H_2O$ (mg/h)) of summer- and winter-collected lizards.

Season	Coefficient	Estimate	Std. Error	DF	t-value	*p*-value
Summer	RMR					
	Intercept	-2.64	0.11	50	-24.14	<0.0001
	TT (°C)	0.05	0.002	50	22.69	<0.0001
	M_b (g)	0.10	0.02	50	5.88	<0.0001
	WLR					
	Intercept	-1.14	3.13	46	-0.36	0.72
	TT (°C)	0.21	0.05	46	4.72	<0.0001
	M_b (g)	1.06	0.50	46	2.12	<0.05
Winter	RMR					
	Intercept	-3.35	0.30	80	-11.31	<0.0001
	ACC (°C)	0.05	0.02	80	3.33	<0.01
	TT (°C)	0.10	0.01	80	8.95	<0.001
	M_b (g)	0.06	0.02	80	2.44	<0.05
	ACC x TT	-0.003	0.0007	80	-3.64	<0.001
	WLR					
	Intercept	-2.11	1.59	79	-1.33	0.19
	TT (°C)	0.31	0.02	79	13.79	<0.0001
	M_b (g)	0.44	0.22	79	2.03	<0.05

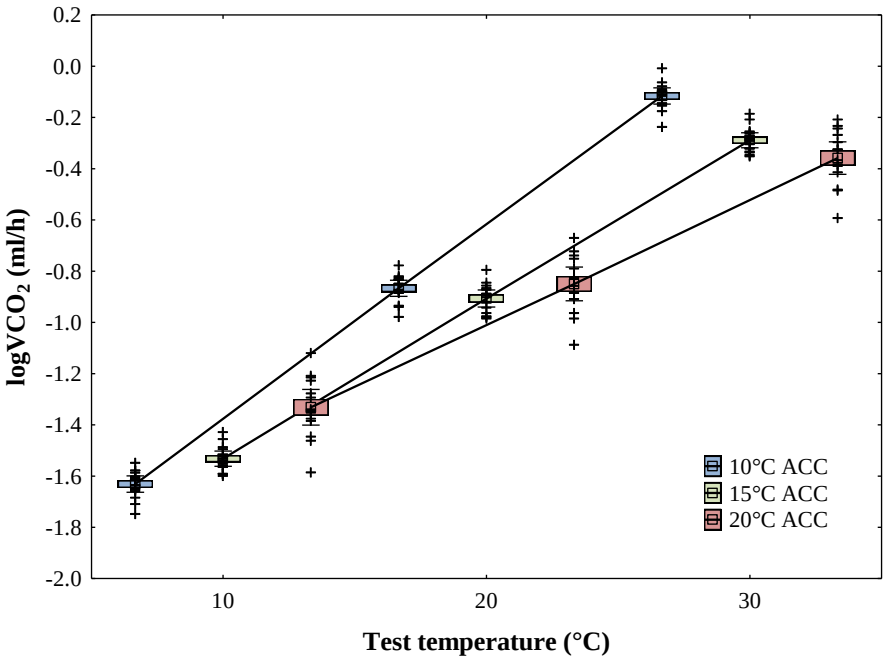

Figure 1.2. Test temperature effects on resting metabolic rate (RMR) in lizards collected in winter and acclimated to three treatments (10, 15 or 20°C). The data shown are estimates from the mixed effects model, boxes represent means ± s.e.m. and whiskers are ± 95% CL. The spread of estimates is shown with '+' symbols. Lizards from each of the acclimation treatments (ACC) were tested at three test temperatures. Boxes for each ACC at each test temperature are separated in the figure to avoid overlap.

Between seasons, mean M_b of male lizards did not differ (6.8 ± 1.1 g and 6.4 ± 1.0 g for winter and summer, respectively; t(46)=-1.37, p>0.05) but female lizards from the winter population were significantly larger than the non-gravid females from the summer population (7.2 ± 1.3 g and 5.5 ± 1.1 g for winter and summer, respectively; t(39)=-3.59, p<0.05). At the TT of 30°C, mass-adjusted RMR was lower in summer than winter and an interaction between ACC and season reflected differences in the slopes of RMR and acclimation groups: no effect of acclimation in summer but an increase in RMR of cold acclimated lizards relative to warm acclimated ones (Table 1.3, Fig. 1.3a). Males had lower RMR at the warmest acclimation treatments while no significant differences were found for RMR in females across treatments (ACC*sex interaction, Table 1.3). Mass-adjusted WLR in winter was significantly lower than in summer lizards and males had higher WLR than females (Table 1.3, Fig. 1.3b). When comparing seasonal differences for individuals exposed to the same 20°C ACC treatment, lizard RMR was not different between seasons but winter lizards had lower WLR than summer lizards (Table 1.4).

Table 1.3. Generalized linear model for the effects of season, acclimation treatment and sex on mass-adjusted RMR and WLR of summer- and winter-collected lizards and measured at 30°C test temperature.

Coefficients	Estimate	Std. error	t-value	*p*-value
RMR				
Intercept	-0.75	0.33	-2.25	<0.05
Season	0.97	0.36	2.71	<0.01
ACC (°C)	0.03	0.01	1.82	0.07
Sex	0.93	0.40	2.31	<0.05
Season x ACC	-0.04	0.02	-2.28	<0.05
Season x Sex	-0.79	0.44	-1.79	0.08
ACC x Sex	-0.04	0.02	-2.16	<0.05
Season x ACC x Sex	0.03	0.02	1.47	0.15
WLR				
Intercept	0.29	0.89	0.33	0.74
Season	-2.35	0.87	-2.71	<0.01
Sex	1.71	0.85	2.01	<0.05

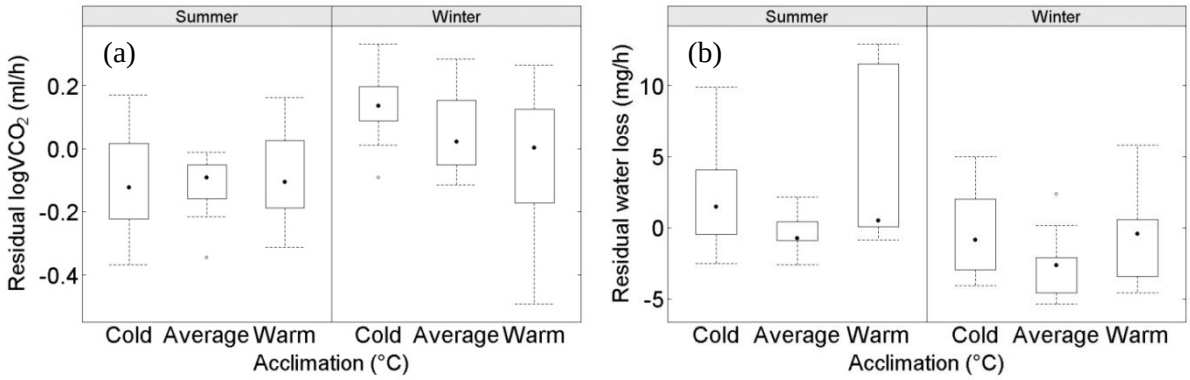

Figure 1.3. Seasonal comparison of (a) RMR and (b) WLR in lizards acclimated at three temperatures (ACC: 'Cold', 'Average' and 'Warm') and measured at 30°C test temperature only. (a) Both Season and the interaction of ACC*Season had a significant effect on RMR (see Table 1.3). (b) Winter-collected lizards had a significantly lower WLR than summer-collected lizards. Boxes represent the interquartile range (IQR) of the data and whiskers are 1.5*IQR when not encompassing the minimum and maximum of the data points.

Table 1.4. General mixed effect model for the effects of season, test temperature, body mass and sex on RMR and WLR in lizards from the 20°C acclimation groups only.

Coefficient	Estimate	Std. Error	DF	t-value	*p*-value
RMR					
Intercept	-2.74	0.14	43	-19.49	<0.0001
Season	0.11	0.06	43	1.81	0.08
TT (°C)	0.05	0.003	43	16.34	<0.0001
M_b (g)	0.12	0.02	43	5.73	<0.0001
WLR					
Intercept	-0.76	2.23	43	-0.34	0.73
Season	-5.02	0.97	43	-5.16	<0.0001
TT (°C)	0.27	0.05	43	5.91	<0.0001
M_b (g)	0.99	0.35	43	2.86	<0.01

1.3.2 Preferred body temperature

In summer, lizards in the warmest acclimation treatment selected the lowest mean and lowest maximum T_{sel} compared to the other two acclimations (Fig. 1.4, Tables 1.5 and 1.6). Mean T_{sel} was 31.5 ± 0.8°C and 33.2 ± 0.6°C, max T_{sel}: 33.3 ± 0.5°C and 35.0 ± 0.8°C for the warm and cold acclimation groups, respectively. M_b and sex did not affect mean or maximum T_{sel} (p>0.05). In winter, none of the predictor variables had an effect on mean, maximum, minimum or range of T_{sel} selected (Fig. 1.4).

Table 1.5. Summary table of the central 50% for T_{sel} of winter and summer lizards acclimated at low, average and high summer and winter conditions. All values (minimum, maximum, range and mean) are in °C ± s.e.m.

Season	ACC (°C)	Sex	Minimum	Maximum	Range	Mean	n
Summer	20	F	32.6 ± 0.5	35.7 ± 0.2	3.0 ± 0.5	34.3 ± 0.2	5
Summer	20	M	31.1 ± 1.1	34.9 ± 0.7	3.7 ± 0.6	33.0 ± 0.9	5
Summer	25	F	31.9 ± 0.5	34.9 ± 0.3	3.7 ± 0.2	33.0 ± 0.4	4
Summer	25	M	30.6 ± 0.4	34.5 ± 0.4	3.9 ± 0.7	32.6 ± 0.2	4
Summer	30	F	30.6	33.4	2.8	32	1
Summer	30	M	30.4 ± 0.7	33.8 ± 0.5	3.4 ± 0.4	32.3 ± 0.5	5
Winter	10	F	29.7 ± 1.3	34.0 ± 0.7	4.4 ± 0.8	32.0 ± 0.91	5
Winter	10	M	29.7 ± 1.4	32.8 ± 1.3	3.1 ± 0.4	31.3 ± 1.3	7
Winter	15	F	29.2 ± 1.6	33.5 ± 0.8	4.3 ± 0.8	31.4 ± 1.2	8
Winter	15	M	30.1 ± 0.8	33.5 ± 0.5	3.4 ± 0.4	31.9 ± 0.6	6
Winter	20	F	31.2 ± 1.1	34.7 ± 0.6	3.5 ± 0.7	33.2 ± 0.7	6
Winter	20	M	30.3 ± 0.8	34.1 ± 0.5	3.8 ± 0.5	32.6 ± 0.6	8

Table 1.6. Generalized linear model for the effects of acclimation (ACC), mass and sex on mean and maximum T_{sel} of summer-collected lizards. Both mean and maximum T_{sel} were significantly lower in lizards from the warm ACC (30°C) compared to the cold ACC (20°C).

Coefficient	Estimate	Std. Error	t-value	*p*-value
Mean T_{sel}				
Intercept	33.60	0.57	59.10	<0.001
ACC 25°C	-1.16	0.83	-1.40	0.18
ACC 30°C	-2.03	0.89	-2.29	<0.05
Maximum T_{sel}				
Intercept	34.55	0.40	87.04	<0.0001
ACC 25°C	-0.55	0.59	-0.92	0.36
ACC 30°C	-1.69	0.64	-2.65	<0.05

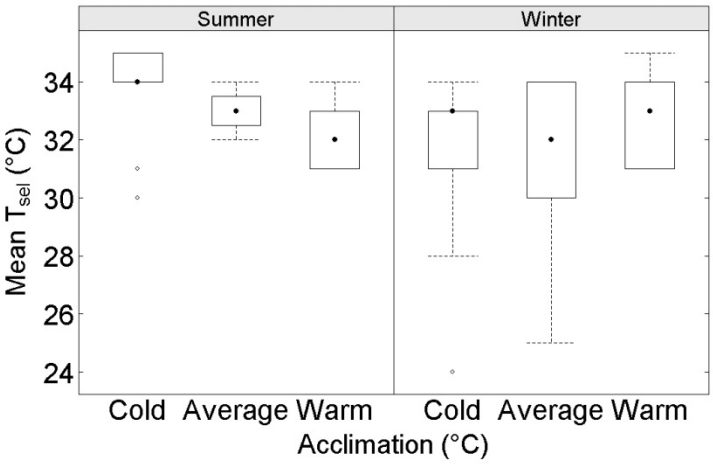

Figure 1.4. Winter-collected lizards selected lower mean T_{sel} than summer-collected lizards but in winter, T_{sel} did not differ across acclimations. Within summer, lizards from the 'Warm' acclimation selected a significantly lower mean T_{sel} than lizards from the other two acclimation treatments. Boxes represent the interquartile range (IQR) of the data and whiskers are 1.5*IQR when not encompassing the minimum and maximum of the data points.

Between seasons, winter lizards selected lower mean (GLZ, t(61)=-2.24, p<0.05, Fig. 1.4) and minimum T_{sel} (t(60)=-2.08, p<0.05) than did summer lizards (all ACC mean T_{sel}: 32.0 ± 0.4°C and 32.5 ± 0.4°C, minimum T_{sel}: 30.5 ± 2.8°C and 31.7 ± 1.7°C for winter and summer, respectively). A significant interaction for mean T_{sel} between season and acclimation (Season*ACC, t(61)=2.05, p<0.05) reflects the difference in the relationship between mean T_{sel} and acclimations for summer versus winter as described previously.

1.3.3 Seasonal lizard activity, thermal constraints and prey availability

From transects, 147 and 597 lizards were observed in winter and summer, respectively. During winter and summer, the majority of lizards were basking, but ± 10% and 12% of lizards were running at time of observation for winter and summer respectively. In winter, 52.4% of lizards were observed between 12:00 and 13:00 (Fig. 1.5a), whereas in summer, lizards were observed over a larger portion of the day: 16.4%, 31.3%, 22.6%, 18.1% and 11.6% of lizards observed between 08:00-09:00, 10:00-11:00, 12:00-13:00, 15:00-16:00 and 18:00-19:00, respectively (Fig. 1.5b). In summer, the average habitat quality index ($\overline{d}_e$) for the warm ACC lizards (12.2 ± 6.6°C) was significantly lower than cold ACC lizards (14.2 ± 6.6°C; $t_{0.05(2),3770}$=-9.28, p<0.05). Winter $\overline{d}_e$ (23.1 ± 4.6°C) was significantly higher than summer ($t_{0.05(2),4070}$=50.60, p<0.05 and $t_{0.05(2),4070}$=61.91, p<0.05 for winter and cold-acclimated summer lizards, and winter and warm-acclimated summer lizards, respectively).

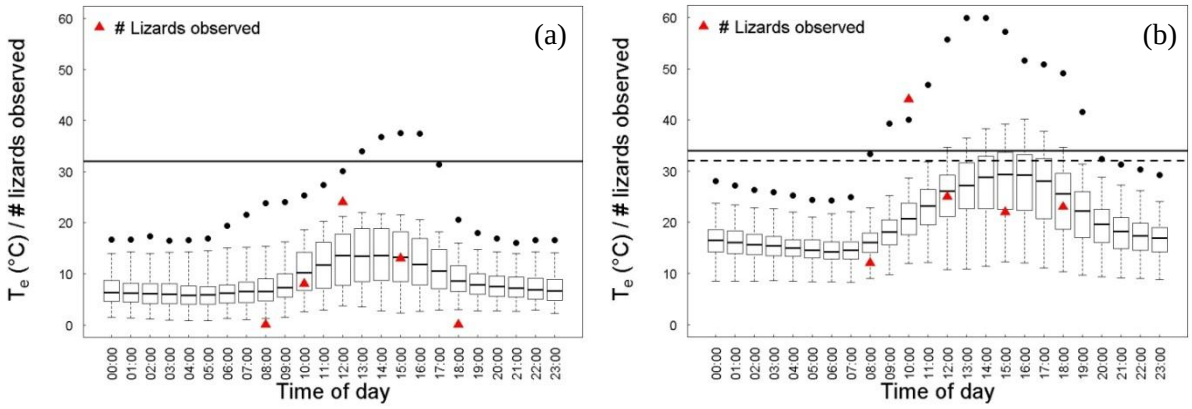

Figure 1.5. Lizard operative temperatures (T_e) in (a) winter (June - August, 2011) and (b) summer (January - March, 2012). Boxes represent median and quartile T_e with the whiskers set at maximum and minimum values of mean T_e for that time of day. Black dots indicate absolute maximum T_e for that time of day. In Fig. a. the solid line is the mean T_{sel} of winter lizards from all three acclimations. The solid and dotted lines in Fig. b. are the mean T_{sel} for summer cold- and warm-acclimated lizards, respectively. Red triangles indicate the number of lizards observed on a sunny day in (a) winter and (b) summer during the transect walks.

Invertebrate species diversity (H` = 2.58 and 2.86 for summer and winter, respectively) and evenness ($E_{1/D}$ = 0.10 and 0.34 for summer and winter, respectively) were lower in summer than winter, but dominance of a single family was higher in summer (d = 0.34 and 0.22 for summer and winter, respectively). Total abundance was 952 individuals (29 taxonomic families) in summer and 207 individuals (25 families) in winter with ants being the most abundant in both seasons (see Table 1.7). Faecal content of *C. oelofseni* at the same site in summer and winter (Clusella-Trullas & Botes 2007) indicates that the prey consumed by this species highly overlaps with the invertebrate groups collected for both seasons (Table 1.7).

Table 1.7. Number of invertebrate prey collected using pitfall traps and net sweeping in summer (2012) and winter (2011) at Landdroskop, in the south-western Cape of South Africa. Data in the last two columns are percentages of prey occurrence for each taxonomic order found in faecal pellets of *C. oelofseni* collected in summer (19 samples) and winter (12 samples) by Clusella-Trullas & Botes (2007).

		This study		Faecal samples	
Order	**Family**	**Winter**	**Summer**	**Winter**	**Summer**
Arachnida	Aranea	13	82		
Coleoptera	Chrysomaelidae	21	110	75%	74%
	Coccinellidae	1			
	Curculionidae	5	172		
	Scarabaeidae		1		
	Tenebrionidae	1	1		
Collembola	Entomobryoidae	1			
Diptera	Asilidae		1		
	Cecidomyiidae	22			
	Empididae	2			
	Muscidae	1	2		
	Mycetophilidae	12			
	Nemestrinidae		3		
	Sciaridae	33			
	Tabanidae		1		
	Tephritidae	2	2		
Hemiptera	Cercopidae	5		33%	5%
	Cicadellidae	7	69		
	Cicadidae		1		
	Dictyopharidae		1		
	Delphacidae	19			
	Issidae	6	5		
	Lygaeidae	2	2		
	Miridae	1			
	Pentatomidae	1	2		
Hymenoptera	Formicidae	67	422	17%	26%
	Ichneumonidae		1		
	Melittidae		2		
	Scoliidae		1		
	Braconidae	2	1		
Isoptera	Termitidae	4			

Lepidoptera	Brachodidae		1		
Mantodea	Mantidae		2		
Orthoptera	Acrididae	3	16		10%
	Anostostomatidae		1		
	Gryllacrididae		1		
	Lentulidae	8	39		
	Tettigoniidae		5		
	Tetrigidae	1	1		
Blattodea					5%
Solfugida					5%
Plant material				17%	10%

1.4 Discussion

Huey & Berrigan (1996) emphasized the need to determine the net benefits of acclimation in natural environments. Nonetheless, few studies report the climatic variation encountered at the scale of the animal studied and how these conditions are taken into account during thermal exposures in the laboratory (see e.g. Šamajová & Gvoždík 2010). Moreover, few studies in vertebrate taxa, especially including reptiles, have provided such in-field assessments. In this study, I integrate ecological reality to responses of several traits to thermal acclimation regimes and find that the precise trait responses of thermoregulation, energetics and water-loss rates can be complex and variable depending on the scale investigated. In the short-term, behavioural rather than physiological compensation takes place in summer while partial compensation of MR appeared to be more significant than behaviour in winter. When lizard responses are compared between seasons, both behavioural and physiological shifts take place: summer lizards select overall lower T_{sel} than winter lizards and when physiological responses were compared at a common temperature near T_{sel} (30°C), summer lizards had lower RMR, but higher WLR than winter lizards.

The variation in trait responses observed in *C. oelofseni* may be interpreted as being beneficial from a fitness perspective. For example, in summer I found that there is a small but significant lowering of T_{sel} in lizards acclimated to the warm treatment (30°C, Table 1.6, Fig. 1.4a). Since T_{sel} is near critical thermal maxima in these (CT_{max} = 40.8 ± 0.5°C for *C. oelofseni*, Clusella-Trullas *et al*. 2009) and other ectotherms, exposure to elevated temperatures for extended periods of time can result in compensation behaviour that reduces the risk of overheating (Huey & Bennett 1990; Martin & Huey 2008; Sinervo *et al*. 2010). A lowering of T_{sel} in warm acclimated lizards has been found in other studies, especially when the acclimation treatments were at or above T_{sel} (Wilhoft & Anderson 1960; see Clusella-Trullas & Chown *in press* for a review). This behavioural shift in *C. oelofseni* is not associated with thermal constraints in their natural habitat (Fig. 1.5b). Indeed, the thermal quality of the habitat is high in summer (low $\overline{d}_e$) and the availability of both warm and cool microsites allows lizards to regulate their T_b relatively easily within safe margins. In this high thermal quality environment, the need to thermoregulate decreases (as T_e approaches T_{sel}) and lizards are free to pursue other activities such as mating and foraging (Hertz *et al*. 1993).

In winter, *C. oelofseni* is highly constrained by the thermal quality of the habitat (high $\overline{d}_e$, Fig. 1.5a) but lizard activity patterns and prey diversity and abundance in the field demonstrate that *C. oelofseni* remains active in winter and has ample resource availability (Table 1.7). The RMR responses documented here follow a partial compensation (‘Precht type 3’ response, Precht 1958): lizards from the cold-acclimated group had elevated RMR at the highest test temperatures, with no compensation at the lowest test temperature (Table 1.2, Fig. 1.2). Increased RMR likely allows *C. oelofseni* to utilize thermal windows of opportunity throughout winter (see e.g. Hare *et al*. 2010). Although the thermal quality of the habitat is low in winter, warm days are scattered throughout the season and these

recurring opportunities, albeit unpredictable, may form part of the information acquired by these organisms. Increased metabolism (perhaps regulated via thyroid hormone, see Little *et al*. 2013) may enable more rapid cellular responses to enhance performance in changing environmental conditions (Clarke & Fraser 2004). Indeed, metabolic cold compensation has been reported for other temperate species that remain active in winter (Roberts 1968; Dutton & Fitzpatrick 1974; Davies *et al*. 1981; Tsuji 1988b). In contrast to RMR, short-term exposure to three temperature regimes in winter did not influence T_{sel} (Fig. 1.4). Despite the limited temperature opportunities in their habitat in winter, these lizards consistently seek 32°C when conditions allow.

In addition to short-term plastic responses, seasonal acclimatisation of all traits was found. RMR in winter was higher than in summer when all acclimation treatments were compared at 30°C (Fig. 1.3a, closest temperature to T_{sel}), while T_{sel} and WLR were lower in winter (Table 1.3, Fig. 1.3b). These responses may reflect distinct energetic demands between seasons. Activity during transient warm weather and usage of available resources in winter may require metabolic compensation and thus, increased costs to optimize energy throughput during cold conditions (i.e. 'increased intake hypothesis', Burton *et al*. 2011). However, lower RMR in summer could also reflect compensation to decrease metabolic costs at high temperatures (Tsuji 1988a), or changes in the partitioning of energy allocation to maintenance, growth and reproduction (Congdon *et al*. 1982; Dunham *et al*. 1989). Similarly, shifts in T_{sel} likely accompany the optimum temperature at which performance and underlying biochemical processes operate. For metabolism, it is difficult to discern between compensatory strategies (i.e. higher RMR in winter or lower RMR in summer) without exploring the relative costs and benefits relating to each strategy (e.g. energy budget including maintenance and production demands) and investigating underlying molecular and cellular mechanisms (see Seebacher 2005). However, this study indicates that these compensatory responses are only apparent when comparing acclimation treatments that are relevant for their respective seasons, as the comparison of short-term exposure to a common temperature of 20°C resulted in similar RMR for both seasons (Table 1.4).

The upward shift in WLR in summer compared to winter (Fig. 1.3b) is puzzling given that this response was counter to that of RMR and that compensation for water-loss would be expected to occur during the driest conditions of the year (Bentley & Schmidt-Nielson 1966; Mautz 1982; Dmi'el *et al*. 1997). However, the population of *C. oelofseni* investigated in this study is restricted to mountain tops where orographic fog is frequent in both summer and winter, with mean daytime relative humidity during summer of 69 ± 21% and winter 70 ± 26% (weather station data from winter 2011 and summer 2012). Therefore, these lizards may have limited scope for restricting water loss in dry and warm conditions. The increased WLR for summer-collected lizards may have resulted from associated traits other than metabolism such as behavioural traits (e.g. panting mechanisms for evaporative cooling; Tattersall *et al*. 2006) or seasonal changes in cutaneous properties (Mautz 1982). The plastic response of WLR to temperature change and its components (cutaneous versus respiratory) in lizards has been

seldom explored and merit further research (Lillywhite 2006, for humidity acclimation see Kobayashi *et al*. 1983; Kattan & Lillywhite 1989). Nonetheless, sex and sex x acclimation effects found for WLR and RMR in seasonal comparisons suggest that other factors such as body condition (fat body stores) and/or reproductive cycles likely contribute to physiological responses and should be incorporated in future work. For example, both spermiogenesis and vitellogenesis are known to be maintained throughout winter for three closely-related species of cordylids (Flemming & van Wyk 1992; van Wyk 1994; van Wyk & Mouton 1998) and energy invested in these mechanisms may contribute to some of the patterns found.

In addition to contrasting responses in the short-term and seasonal scales, I found considerable variation depending on the trait examined. By contrast to RMR and T_{sel} responses, WLR responses appear less flexible across the full range of treatment conditions investigated here, irrespective of season. These results therefore highlight the importance of incorporating both behavioural and physiological plasticity in models that predict ectotherm response to climate change. Most likely, the challenge will be to integrate (and translate into a common currency) the magnitude, direction and fitness consequences of plastic responses for multiple traits that may underlie competing functions, in order to predict the consequences of global environmental change.

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Chapter 2

Energetics of the lizard *Cordylus oelofseni*: a test of the cost-benefit model of thermoregulation

2.1 Introduction

Most lizards strive to maintain their body temperature (T_b) within a relatively narrow range of temperature that optimizes physiological and biological functioning, including growth, metabolism, locomotion, reproduction and survival (Beauchat 1986; Adolph 1990; Cadena & Tattersal 2009; Angilletta *et al*. 2010; Besson & Cree 2010). The optimal temperature (T_{sel}) is mostly maintained through behavioural thermoregulation, by seeking adequate microsites in space and time (Huey 1974; Blouin-Demers & Weatherhead 2002). While this is true for most lizards, including basking, fossorial, diurnal and nocturnal species (Bauwens *et al*. 1996, 1999; Christian 1998; Hitchcock & McBrayer 2006; Bowker *et al*. 2010), some species do not thermoregulate and instead thermoconform with the environment (Ibargüengoytía *et al*. 2010; Sinervo *et al*. 2010) or abandon thermoregulation under particular conditions or in certain habitats (e.g. tropical species Huey 1974; Huey & Webster 1976; Hertz *et al*. 1993). As the regulation of T_b within the optimal range has direct consequences for the survival and fitness of these organisms, understanding what strategy (thermoregulation, thermoconformation or an intermediate strategy) is favoured under which conditions is essential for predicting species' ability to buffer climate variation and respond to climate change.

Huey & Slatkin (1976) proposed a conceptual model that predicts the amount of thermoregulation expected given the thermal quality of the environment, based on costs (energy expenditure, mortality risks and loss of foraging/mating opportunities) and benefits (maximizing net energy gain) associated with thermoregulation (see Fig. 2.1 for the cost-benefit model of Huey & Slatkin 1976 and extension of the model by Vickers *et al*. 2011, referred to as 'the CB model' hereafter). According to this model, lizards should thermoregulate carefully only when the associated costs are low. Costs are expected to be low in high quality thermal habitats where the abundance of optimal microsites lowers the distance needed to travel in order to find basking sites, lowers the risks of predation and increases mating opportunities. By contrast, in low thermal quality habitats, lizards should thermoconform rather than thermoregulate because the costs are expected to become too high. However, despite several tests in the field and in controlled laboratory settings, data in support of the model have been mixed.

In general, field-studies conducted in extreme climatic locations do not support the CB model. For example, two species of snakes (*Elaphe obsoleta obsoleta* and *Lampropeltis triangulum*) at the northern extreme of their range in Canada invest more energy in thermoregulation despite the potential costs in their thermally challenging environment (Blouin-Demers & Weatherhead 2001; Blouin-Demers & Weatherhead 2002; Row & Blouin-Demers 2006). These authors suggest that the costs involved with thermoconformation (e.g. inability to digest food or move) are higher than the costs of thermoregulation and thus, more energy should be invested in elevating T_b, if only for short periods of time during the day (Blouin-Demers & Weatherhead 2001). Similarly, the lizard *Zootoca vivipara* in the sub-Arctic invests substantial energy in thermoregulation and is an effective thermoregulator despite its challenging thermal environment (Herczeg *et al*. 2003). However, two lizard species of

Liolaemus that inhabit the cold Patagonia region of Argentina are poor thermoregulators even when short windows of thermal opportunity occur) and thus, support the CB model (Ibargüengoytía *et al*. 2010). These authors suggest that these species may thermoconform as a result of phylogenetic inertia, not having evolved appropriate thermoregulatory behaviours to deal with the extreme thermal challenges of their environment, as their ancestors lived in lower and warmer latitudes.

By contrast to studies undertaken in challenging climates, field studies in the tropics often support the CB model. Shine & Madsen (1996) argued that most tropical reptiles are not restricted by the thermal environment and can deprioritise thermoregulation in favour of other activities such as mating or foraging. For example, *Anolis gundlachi* in Puerto Rico does not actively thermoregulate, but maintains constant high T_b by simply inhabiting a thermally homogeneous habitat, with limited thermal constraints (Hertz *et al*. 1993). For *Anolis cristatellus*, lizards thermoregulate less carefully in shaded forests than adjacent open parks, likely as a result of greater distances and thus, higher costs, between basking sites in the forest (Huey 1974). These results support the idea that as costs increase, thermoregulatory effort decreases, and are in line with the CB model. However, when examining the behaviour of lizards faced with thermal conditions above (as opposed to below) their preferred body temperature, Vickers *et al*. (2011) found that tropical lizards thermoregulate with more precision in low quality habitats due to the risk of approaching lethal temperatures. These contrasting results are not limited to field-studies. For example, under controlled conditions, cold-adapted *Sphenodon punctatus* from New Zealand thermoregulates more accurately when placed in a low-quality habitat (T_{sel} available for 3 hrs a day) compared to a high-quality habitat (T_{sel} available for 8 hrs a day), a result that conflicts with the predictions of the CB model (Besson & Cree 2010). By contrast, *Zootoca vivipara* lizards changed from thermoregulation to thermoconformation when placed in a low temperature treatment with reduced opportunity to reach their preferred body temperature, showing experimental support for the CB model (Herczeg *et al*. 2006). The mixed support found in earlier studies indicates that it is difficult to disentangle the relative costs and benefits of thermoregulation, and perhaps a more direct measure relating to fitness may clarify these mechanisms. To my knowledge, only Campbell (1985) attempted to quantify the energetic cost of thermoregulation in a controlled laboratory setting, finding no difference in energy consumption between low- and high-quality treatments. However, in these experiments, lizards were forced to shuttle between heat sources very frequently (every 45 – 180 s) and active metabolic rate data were taken from mammals of similar size.

This study aims to test the primary prediction of the CB model (i.e. 'in low quality environments, energetic costs should increase and less time should be devoted to thermoregulation compared to high quality environments') by directly quantifying the energetic costs of thermoregulation of the lizard *Cordylus oelofseni* in controlled laboratory settings that differ in thermal habitat quality. By using laboratory settings, lizards are exposed to different consequences of thermoregulation given contrasting spatial distributions of operative temperatures (i.e. low versus high number of available

optimal operative temperatures (T_e) and homogeneous versus heterogeneous T_e distributions), but other costs such as risk of predation, time not allocated to foraging or reproduction are non-existent. In addition, I examine whether body mass influences the degree of thermoregulation under different thermal quality scenarios.

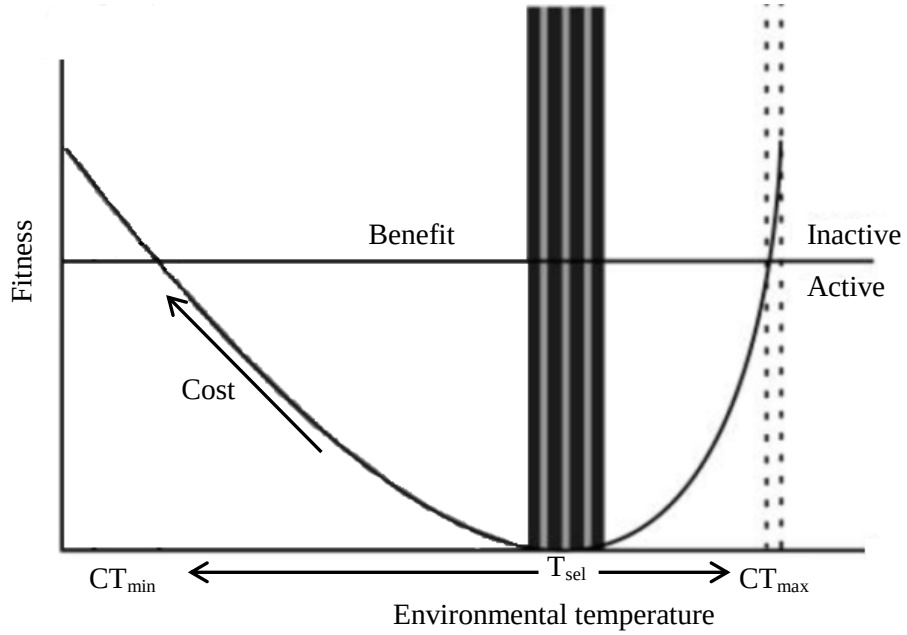

Figure 2.1. The cost-benefit model of thermoregulation describes the optimum amount of thermoregulation (area under the curve) as a function of the costs, with constant gross benefits (Huey & Slatkin 1976, model extended at warm temperatures by Vickers *et al*. 2011). When environmental temperature approaches T_{sel}, the net benefit is maximised and the costs of thermoregulation are nullified. As environmental temperature departs from T_{sel}, the costs of thermoregulation rapidly increase until there is no net benefit (where cost line crosses benefit line) and thermoconforming becomes a better option. At the critical thermal maximum (CT_{max}) and minimum (CT_{min}), activity ceases. Vickers *et al*. (2011) extended the cost-benefit model for tropical lizards to include conditions where T_e is greater than T_{sel}. In this more homogeneous environment, tropical lizards are more effective thermoregulators when T_e is greater than T_{sel}, because of the risks associated with being near CT_{max}.

2.2 Methods and materials

2.2.1 Lizard maintenance and experimental trials

Cordylus oelofseni is a small (6.9 ± 0.8 g, n = 13 males) melanistic, temperate species found in the Hottentots Holland Mountains (*ca.* 1200 m ASL) in South Africa. Seven adult males were collected in November 2011 and 2012 and housed in terrariums (plastic containers with mesh tops, 9.5 L, max. four individuals per container) with stone/sand substrate and artificial refuges and placed within a temperature-controlled incubator (Sanyo Cooled Incubator, MIR-254, Sanyo Electric Co., Ltd., Japan). Lizards were allowed to acclimate to the average operative temperature in the field for this

time of the year (fluctuating temperature cycle, 20/10°C, day/night; 12h:12 photoperiod) for 1 week. Food (crickets and superworms dusted with a vitamin supplement) was provided twice a week and water provided *ad libitum*.

A thermoregulatory enclosure was constructed inside a temperature-controlled room and consisted of four plywood walls (1.8 x 1.8 x 1.0 m) that gave sufficient floor space to include optimal and suboptimal microsites. Light was provided homogeneously across the enclosure from a single fluorescent light bulb fixed in the ceiling. The floor of the arena was divided into 5 x 5 cm squares with sticky tape placed on the floor (Fig. 2.2a). A total of nine basking infra-red (IR) lights (175 W) were equally spaced throughout the arena to create a 3 x 3 matrix of lights and suspended *ca*. 30 cm from the floor (Fig. 2.2b). Nine copper models (same size, shape and reflectance as *C. oelofseni*, see Clusella-Trullas *et al*. 2009 for details) placed directly under each IR light were used to ensure that operative temperatures (T_e) at these microsites were 40 ± 1°C.

The enclosure was used to temporally and spatially manipulate heat sources to simulate four different thermal quality habitats (hereafter referred to as 'enclosure types'): (1) low-quality homogeneous (LQ homog), (2) low-quality heterogeneous (LQ heterog), (3) high-quality homogeneous (HQ homog) and (4) high-quality heterogeneous (HQ heterog). The LQ homog was simulated by setting the climate room to 15°C and leaving all of the IR lights switched off. In this habitat, the T_{sel} of *C. oelofseni* (i.e. 32.6 ± 1.8°C, taken from the average T_{sel} of summer- and winter-collected male lizards acclimated at 20°C as there were no significant differences between seasons at this acclimation temperature, generalized linear model $t(12)=-0.29$, $p>0.05$, see Chapter 1) was unattainable. For the LQ heterog, a single light was selected at random and switched on every hour, for the full hour. In this enclosure type, T_{sel} was only attainable by shuttling between single lights when lamps were alternated (see Fig. A.1 for T_e profile). For the HQ homog, heaters were used to set the room temperature to 30°C. This enclosure type allowed lizards to be near T_{sel}. For the HQ heterog, four IR lights were switched on and alternated every hour instead of one as in the LQ heterog. In HQ heterog, T_{sel} was again attainable by shuttling between lights, but distances between optimal microsites were shorter and basking opportunities more abundant compared to the LQ heterog. All trials occurred between 10:00 to 16:00 daily, with a single lizard being randomly exposed to the four enclosure types on four separate days (as this was randomised, a few lizards could in theory be exposed to enclosures on consecutive days). The lizards were returned to the acclimation treatment between experiments. Lizards were allowed to explore the enclosure for 4 hrs at room temperature (~20°C) during the week of acclimation and were fasted for 24 hrs prior to trials. Water was provided *ad libitum* in the four corners of the arena *ca*. 10 cm from the edges. Each enclosure trial was filmed (Chat Pack CNR-CP12, Canyon) and videos analysed to obtain for each individual trial: total distance travelled and time spent travelling in all enclosures, and time spent basking under a light (i.e. lizard located within a 20 x 20 cm area under the light or demonstrating typical basking behaviour (flattening of body, resting close to the switched-on light) and resting away from the lights (i.e. lizards outside the 20 x 20 cm light areas and not moving)

in the heterog enclosures. Each lizard was weighed before and after each trial (Analytical balance, ± 0.0001 g, AX504, Mettler Toledo International Inc.).

Lizard copper models were used to map the thermal environment by recording T_e every 5 min using a datalogger (CR 1000, Campbell Scientific, Utah, USA). The variation of T_e across the 6 hrs experimental period for the same enclosure type was minimal and <0.5°C. The enclosure was subdivided in nine grids (0.6 x 0.6 m), each corresponding to one light at the centre of the grid (Fig. 2.2a). First, a detailed monitoring of T_e in the grids with lights turned on was undertaken to describe T_e below and away from the IR light by placing one model underneath the light and one every 5 cm away from the light, up to 30 cm (n = 7 models). Second, 13 models were randomly distributed in the remaining enclosure to map T_e in the cold areas (n = 13 models). To calculate mean T_e for the LQ heterog enclosure, seven T_e data points were representative of the warm grid and 56 T_e data points (7 x 8 remaining grids) were representative of the cold grids. In the HQ heterog enclosure, 28 (7 x 4 grids) T_e points represented the warm grids while 35 (7 x 5 grids) T_e data points represented the cold grids. In the homog enclosures, a total of 63 (7 x 9 grids) T_e data points from 20 copper models randomly placed during these treatments were used to map the thermal environment. The combined T_e data from all of the models averaged across experimental time (6 hrs at 5 min sampling intervals) within an enclosure type was used to calculate the mean T_e for each enclosure type.

Habitat quality ($\overline{d}_e = T_{sel} - T_e$) and thermoregulatory accuracy ($\overline{d}_b = T_{sel} - T_b$) for each individual trial and enclosure type were calculated following Hertz *et al*. (1993). $\overline{d}_e$ was calculated as the absolute deviation of T_e from mean T_{sel} (i.e. 32.6°C), resulting in a mean $\overline{d}_e$ per enclosure type. For $\overline{d}_b$, T_b for each enclosure type was calculated by assuming that T_b equalled T_e across the enclosure, and by determining the proportion of time spent by each lizard underneath and away from switched-on lights throughout the 6 h trial (video analysis). The 'area under the light' was assumed to be within a 20 x 20 cm area under the light. From copper model readings, T_e for the LQ heterog enclosure under the light and away from the light was 40.2°C and 16.1°C, respectively, and 40.2°C and 17.9°C for the HQ heterog enclosure. These differences reflect the warmer T_e experienced in cold grids of the enclosure when four lights were turned on simultaneously in the HQ heterog versus one single light in the LQ heterog. T_b in the homogeneous enclosures was assumed to equal T_e (16.3 ± 0.3°C in the LQ homog enclosure and 30.4 ± 0.5°C in the HQ homog enclosure) and seven random draws from T_e datasets from the homogeneous enclosures were taken to simulate random movement of lizards in these enclosures. The absolute deviations of mean T_b from T_{sel} provided individual $\overline{d}_b$ and a mean $\overline{d}_b$ was calculated for each enclosure type. The comparison of $\overline{d}_b$ and $\overline{d}_e$ provides an estimate of thermoregulatory effort. A $\overline{d}_b < \overline{d}_e$ means that lizards select microsites that result in body temperatures closer to T_{sel} compared to random movements which will approximate $\overline{d}_e$. In addition, the effectiveness of thermoregulation ($E = 1 - (\overline{d}_b / \overline{d}_e)$) for each enclosure type and individual trial were calculated following Hertz *et al*. (1993) using $\overline{d}_e$ and $\overline{d}_b$ (Blouin-Demers & Nadeau 2005; Hitchcock

& McBrayer 2006; Row & Blouin-Demers 2006). An *E* that approaches 1 indicates that lizards are careful thermoregulators.

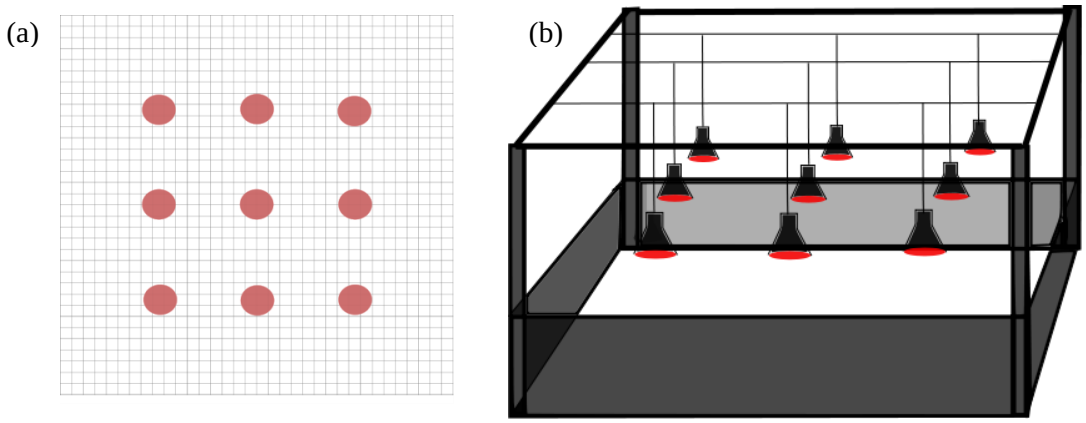

Figure 2.2. View of the thermoregulatory enclosure from (a) the top, showing the floor layout of the enclosure, divided into 5 x 5 cm squares. The red dots indicate where the infra-red lights were suspended. (b) Side-view of the enclosure showing the 9 infra-red lights suspended from wooden beams *ca*. 30 cm from the floor.

2.2.2 Energetic costs

Resting (RMR) and active (AMR) metabolic rate of *C. oelofseni* in combination with activity patterns were used to quantify the overall energetic cost of thermoregulation in each enclosure type. Resting MR data ($mlCO_2/g/h$) of adult males were taken from Chapter 1 and consisted of data from summer- and winter-collected male lizards acclimated at 20°C as no significant differences were found between these two groups at this acclimation temperature (general mixed effect model with test temperature, season and mass as fixed factors and individual as random effect, season effect: $t(23)=0.63$, $p>0.05$). Active MR data ($mlCO_2/g/h$) were obtained from male and female summer-collected lizards (acclimated to 25/15°C, 14h:10 h light cycle, n = 7), exercised on a custom built motorised treadmill. The treadmill consisted of a rubber belt (50 cm in length) rotated by a geared DC motor (45rpm, 24V, RS Components, Midrand, South Africa) around two axes and placed within a Perspex chamber (total volume of chamber = 573.7 ml) with a removable lid (see Fig. A.2a). The treadmill speed was regulated with a panel mount motor speed regulator (6-15V, RS Components) and kept constant at 0.22 ± 0.009 m/s. The front end of the chamber was coated black so as to encourage movement of lizards towards the darkened "crevice". The treadmill set-up was placed within a temperature-controlled incubator (Sanyo Cooled Incubator, MIR-153, Sanyo Electric Co., Ltd., Japan) set at four test temperatures: 15, 20, 25, 30°C. Temperatures inside the chamber were verified using a thermocouple and temperature recorder (TC 1000, Sable Systems, Las Vegas, Nevada, USA) and kept within 1°C of the target temperature. The chamber was connected to a calibrated LI-COR infra-red CO_2/H_2O analyser (Li-7000, LI-COR Inc., Lincoln, Nebraska, USA) set in gas flow configuration and plumbed in differential mode (Lighton 2008). Air was supplied using an air pump (OPTIMA, Hagen

Air Pumps, Durban, SA) which flowed through two scrubber columns (silica gel:drierite (50:50) for scrubbing water and soda lime for scrubbing CO_2). Thereafter the gas flowed through a mass flow control valve (Sierra Instruments, Side-Track Model 840, Monterey, California, USA) controlled by a mass flow control unit (Sable Systems, Intelligent Mass Flow Control Unit, MFC-2, Las Vegas, Nevada, USA) at a rate of 150 ml/min (measured using a hand-held flow meter, ADM 1000, Agilent Technologies, Santa Clara, US). The air then flowed into the LI-COR which measured VCO_2 in differential mode. Before and after lizards were placed in the chamber, VCO_2 base-line recordings were taken for 5 min. After the initial baseline, a single lizard was placed in the treadmill and allowed 10 min to equilibrate to the test temperature. The lid of the chamber was sealed using vacuum grease and petroleum jelly. During this 10 min period, the chamber was flushed with the supplied air until CO_2 levels reached a steady state. The treadmill was then activated by slowly increasing the speed of the treadmill from 0 to 0.22 m/s and the latter speed was similar to the average walking speed of lizards in the experimental enclosures (0.30 ± 0.10 m/s, n = 28). The lizard was encouraged to run on the treadmill by lightly tapping the sides of the chamber and was kept in the chamber until it ran steadily for a minimum of one minute (see Fig. A.2b). Runs were discarded if the lizard made no attempt at running on the treadmill, or ran for less than one minute. Each lizard was run once at each test temperature and weighed before and after each trial. A linear relationship was fitted to the RMR data of males versus test temperature (10, 20 and 30°C) and the linear equation (see addenda, Fig. A.3) was used to obtain RMR from the body temperatures of lizards in each enclosure. For AMR, no differences were found between males and females (general mixed effects model with test temperature, sex and mass as fixed factors and individual as random effect, sex effect: $t(5)=-2.53$, $p>0.05$) and all data were used to obtain the equation describing the temperature dependence of AMR (see addenda, Fig. A.4). For each enclosure type, MR data was converted to $mlCO_2/h$ by multiplying by the mass of the individual lizards used in the enclosures and by the time spent active and resting. Metabolic data were then converted to mlO_2 (assuming a respiratory quotient of 0.7 for fasted animals) and then to Joules ($20J/mlO_2$, Congdon *et al*. 1979; Bennett 1982; Gessaman & Nagy 1988; Sears 2004) for each enclosure type trial.

2.2.3 Statistical analyses

Mean T_e and $\overline{d}_e$ data distributions for heterogeneous enclosures were heavily skewed as they were dependent on the light configuration for each enclosure type. Therefore, the response variables (T_e or absolute $\overline{d}_e$) were log transformed before running a generalised linear model (GLZ, with Gaussian family and identity link function) to test for differences in mean T_e and $\overline{d}_e$ across enclosure types. To test for the effects of enclosure type on thermoregulatory responses, general mixed effects models (nlme package, Pinheiro *et al*. 2013) were used with thermoregulatory accuracy index ($\overline{d}_b$), distance travelled, time spent travelling (i.e. all movements), time spent basking, time spent resting away from lights, or energy consumed used as dependent variables, enclosure type as the fixed predictor and

lizard (individual) as a random predictor. Mixed effects models were compared to generalised least squares (gls) models to verify that models with a random intercept were better models for each response variable (following protocol of Zuur *et al*. 2009) and model validation graphs (e.g. model residuals versus fitted values) were used to check for violation of model assumptions. For ($\overline{d}_b$), a gls model with a varIdent variance structure (nlme package) was a better model than a mixed effects model. The varIdent variance structure was used due to the heterogeneity of the data (differences in the variance of the response variable across enclosure types). Given that the individual effect was significant for most response variables, body mass could not be included in the mixed effects model as mass is confounded by individuals. Therefore, simple linear regressions were used to test for mass effects on response variables within each enclosure type and homogeneity-of-slopes tests were used to identify differences in slopes across enclosure types. All statistical results focus mainly on the comparisons between 'LQ homog and LQ heterog', 'HQ homog and HQ heterog' and 'LQ heterog and HQ heterog' enclosure types. All statistical analyses were conducted in R (v. 2.15.0, 2012, R Development Core Team). A significance level of $\alpha < 0.05$ was set for all statistical tests. All results presented in Tables are minimal adequate models based on Akaike Information Criteria (Burnham & Anderson 2004) and general mixed effects models were compared using maximum likelihood (Crawley 2007; Zuur *et al*. 2009). Means are reported ± s.d. unless stated otherwise.

2.3 Results

Both heterogeneous enclosures had higher T_e variation than the homogeneous enclosures due to light configurations (Fig. 2.3). Mean T_e in the HQ heterog was lower than in the HQ homog enclosure ($t(251)=10.95$, $p<0.001$) and higher than the LQ heterog enclosure ($t(251)=-7.81$, $p<0.001$). Mean T_e did not differ between the LQ heterog and LQ homog enclosures ($t(251)=1.62$, $p=0.1$, Table 2.1). Mean $\overline{d}_e$ was higher in the HQ heterog enclosure than the HQ homog enclosure ($t(251)=-17.69$, $p<0.001$) but lower than the LQ heterog enclosure ($t(251)= 5.17$, $p<0.001$). $\overline{d}_e$ did not differ between LQ heterog and LQ homog enclosures ($t(251)= -1.35$, $p>0.05$, Table 2.1, Fig. 2.4a).

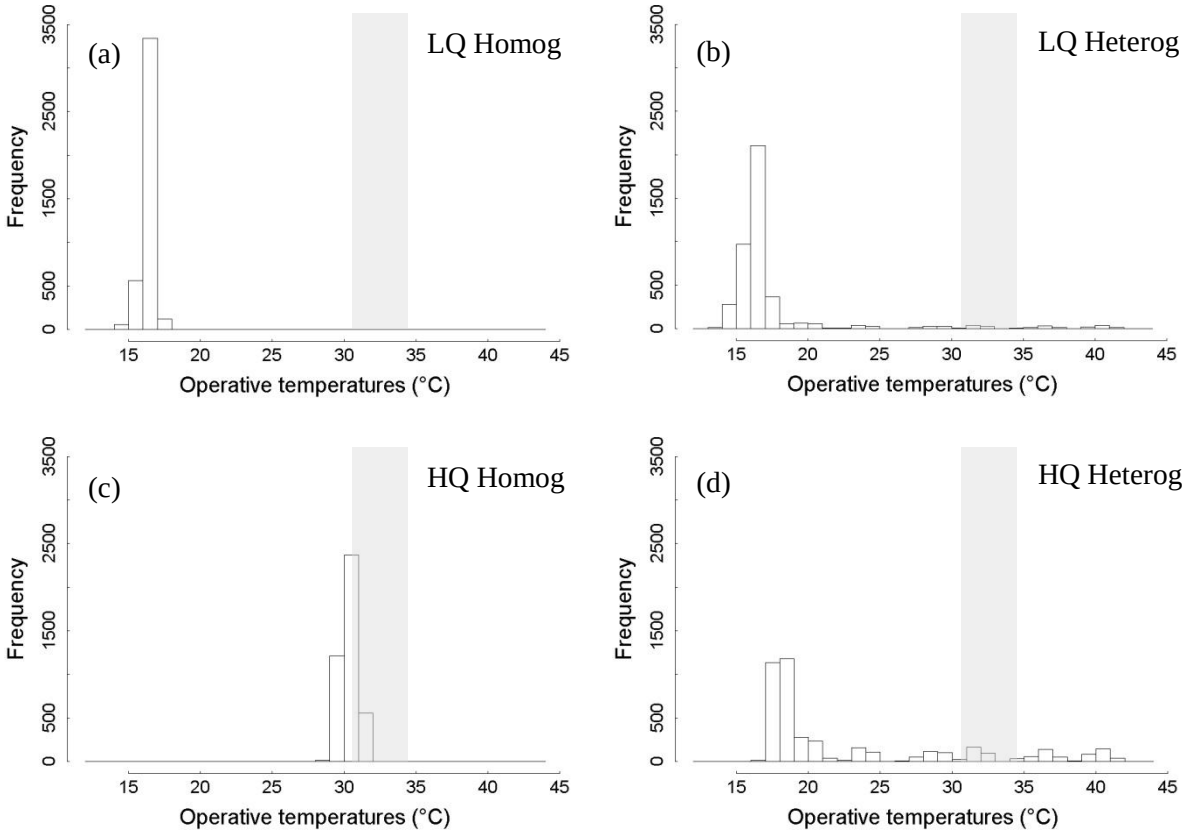

Figure 2.3. Frequency distributions of operative temperatures (T_e) available in each of the enclosure types during 6 h trials (sampled at 5 min intervals, see methods): (a) low quality homogeneous (LQ homog), (b) low-quality heterogeneous (LQ heterog), (c) high-quality homogeneous (HQ homog) and (d) high-quality heterogeneous (HQ heterog). Grey shaded bars incorporate the mean $T_{sel} \pm$ s.d.

The index of thermoregulatory accuracy $\overline{d}_b$ was significantly lower in the HQ homog enclosure than in the HQ heterog enclosure (Tables 2.1 and 2.4, Fig. 2.4b) whereas $\overline{d}_b$ was significantly higher in the LQ homog enclosure compared to the LQ heterog enclosure ($t(28)=12.42$, $p<0.001$). $\overline{d}_b$ did not differ

significantly between the LQ heterog and HQ heterog enclosures (Table 2.4, Fig. 2.4b). The efficiency of thermoregulation index, *E*, was significantly higher in the LQ heterog enclosure than in the HQ heterog enclosure (paired t-test, t(6)=5.38, $p<0.01$, Table 2.1).

Table 2.1. Summary of mean operative temperatures (T_e) and thermoregulatory indexes (thermal habitat quality $\overline{d}_e$, thermoregulatory accuracy $\overline{d}_b$ and efficiency of thermoregulation *E*) for each enclosure type: low-quality homogeneous (LQ homog), low-quality heterogeneous (LQ heterog), high-quality homogeneous (HQ homog) and high-quality heterogeneous (HQ heterog). Note that samples sizes for T_e and $\overline{d}_e$ are 63 copper models per enclosure type whereas Mean $\overline{d}_b$ and *E* are calculated for 7 lizards per enclosure type.

	LQ homog	LQ heterog	HQ homog	HQ heterog
Mean T_e (°C)	16.4 ± 0.3	17.6 ± 4.7	30.4 ± 0.5	22.7 ± 7.3
Mean $\overline{d}_e$ (°C)	16.2 ± 0.3	15.4 ± 3.3	2.2 ± 0.5	11.3 ± 4.7
Mean $\overline{d}_b$ (°C)	16.2 ± 0.04	11.0 ± 1.1	2.3 ± 0.1	10.2 ± 0.2
E		0.28 ± 0.1		0.10 ± 0.02

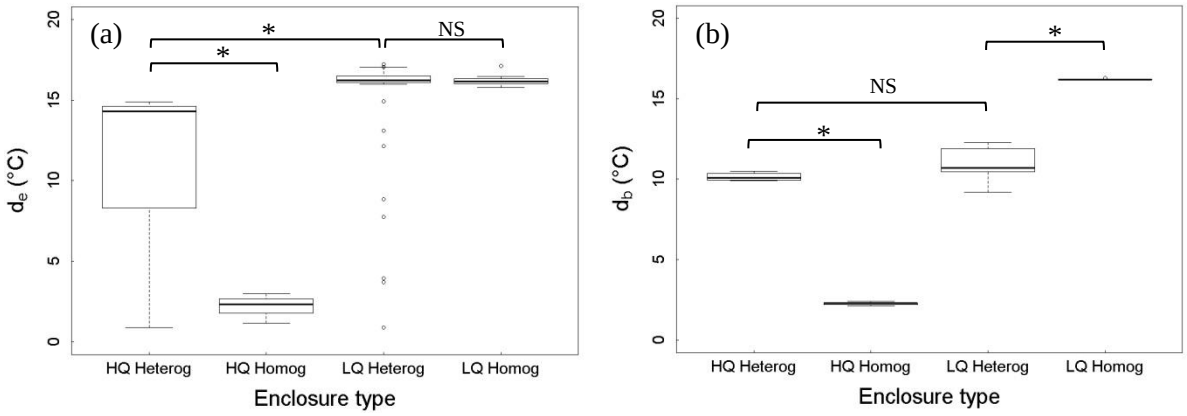

Figure 2.4. (a) Thermal habitat quality index ($\overline{d}_e$) calculated from a total of 63 microsites in each enclosure type. A lower $\overline{d}_e$ reflects enclosures with higher thermal quality as the available T_es are closer to T_{sel}. (b) Thermoregulatory accuracy index ($\overline{d}_b$) calculated from 7 male lizards in each enclosure type. Lower $\overline{d}_b$ indicates higher accuracy of thermoregulation. Upper black lines indicate statistical comparisons between enclosures (* = significant differences at $p<0.05$; NS = non-significant). Boxplots provide the median (black horizontal line), the inter-quartile range (upper and lower sides of the box) and min and max values excluding outliers (whiskers).

For total distance travelled, lizards from the homog enclosures moved significantly shorter distances than lizards from the heterog enclosures (Tables 2.2 and 2.4, Fig. 2.5a; for the LQ homog and LQ heterog comparison: t(28)=-2.58, $p<0.05$) but lizards from the HQ heterog enclosure moved a greater distance than lizards in the LQ heterog enclosure (Tables 2.2 and 2.4, Fig. 2.5a).

For time travelled by the lizards in each enclosure, there was no significant differences in time spent moving between the HQ homog and HQ heterog enclosures (Tables 2.2 and 2.4, Fig. 2.5b) or between the LQ homog and LQ heterog enclosures ($t(28)=-0.44$, $p>0.05$). However, lizards in the HQ heterog enclosure spent more time travelling than those in the LQ heterog enclosure (Tables 2.2 and 2.4, Fig. 2.5b).

Time spent basking between lizards in the LQ heterog and HQ heterog enclosures (61.9± 12.5% and 63.8± 3.3%, respectively) did not differ (Tables 2.2 and 2.4). Similarly, there was no significant difference in time spent resting away from the light between lizards in these two enclosure types (33.6 ± 12.1% and 29.4 ± 3.8% for the LQ heterog and HQ heterog enclosures, respectively, Tables 2.2 and 2.4)

Total energy consumed was higher in the HQ heterog enclosure than in the HQ homog enclosure (Tables 2.2 and 2.4, Fig. 2.5c). Similarly, lizards in the LQ heterog enclosure spent more energy than lizards in the LQ homog enclosure ($t(18)=-12.15$, $p<0.0001$). There was no significant difference in energy consumption between lizards in the LQ heterog and HQ heterog enclosures.

Table 2.2. Summary table of original data from lizards (n = 7) in the four thermoregulatory enclosure types. Variables investigated include total distance travelled (m), time travelled (min, included all movements in the enclosures), time basking (min), time resting (min, time not moving and away from lights) and total energy used (J). Time basking and resting away from lights are for heterogeneous enclosures only. All values are given as mean ± s.e.m.

	LQ homog	LQ heterog	HQ homog	HQ heterog
Distance travelled	140.3 ± 30.8	313.4 ± 58.9	384.4 ± 77.7	581.0 ± 55.8
Time travelled	14.5 ± 3.5	16.0 ± 1.7	19.0 ± 3.7	24.6 ± 1.9
Time basking		223.0 ± 17.0		229.6 ± 4.4
Time resting		121.0 ± 16.5		105.8 ± 5.1
Energy consumed	27.2 ± 1.7	83.5 ± 8.8	78.2 ± 5.8	88.0 ± 5.6

Table 2.3. Percentage of energy consumed (mean ± s.d. %) for each activity observed in the experimental enclosures: locomotion, resting (away from the light in the heterogeneous enclosures) and basking.

Enclosure type	Locomotion	Resting	Basking
LQ homog	5.3 ± 3.4	94.7 ± 3.4	
LQ heterog	2.0 ± 0.7	11.2 ± 5.3	86.9 ± 5.7
HQ homog	4.1 ± 2.1	95.9 ± 2.1	
HQ heterog	3.1 ± 0.6	11.1 ± 1.8	85.8 ± 1.7

Table 2.4. Effects of enclosure type on the accuracy of thermoregulation index ($\overline{d}_b$), distance travelled (m), time spent travelling (min), time basking (min), time resting (away from lights, min) and energy consumption (J) of *Cordylus oelofseni*. Statistical models differ between $\overline{d}_b$ and the other response variables examined (see methods for details).

Coefficient	Estimate	Std. Error	DF	t-value	*p*-value
Mean $\overline{d}_b$					
Intercept	10.16	0.09	28	110.15	<0.0001
HQ homog	-7.90	0.10	28	-79.54	<0.0001
LQ heterog	0.83	0.43	28	1.92	0.07
LQ homog	6.06	0.09	28	64.91	<0.0001
Distance travelled					
Intercept	580.98	58.25	18	9.97	<0.0001
HQ homog	-196.59	67.04	18	-2.93	<0.01
LQ heterog	-267.58	67.04	18	-3.99	<0.001
LQ homog	-440.66	67.04	18	-6.57	<0.0001
Time travelled					
Intercept	24.57	2.86	18	8.60	<0.0001
HQ homog	-5.58	3.36	18	-1.66	0.11
LQ heterog	-8.59	3.36	18	-2.56	<0.05
LQ homog	-10.07	3.36	18	-3.00	<0.01
Time basking					
Intercept	229.62	12.44	6	18.46	<0.0001
LQ heterog	-6.64	17.59	6	-0.38	0.72
Time resting					
Intercept	105.81	12.23	6	8.65	<0.001
LQ heterog	15.22	17.29	6	0.88	0.41
Energy consumed					
Intercept	88.03	6.01	18	14.64	<0.0001
HQ homog	-9.83	4.63	18	-2.12	<0.05
LQ heterog	-4.52	4.63	18	-0.98	0.34
LQ homog	-60.79	4.63	18	-13.12	<0.0001

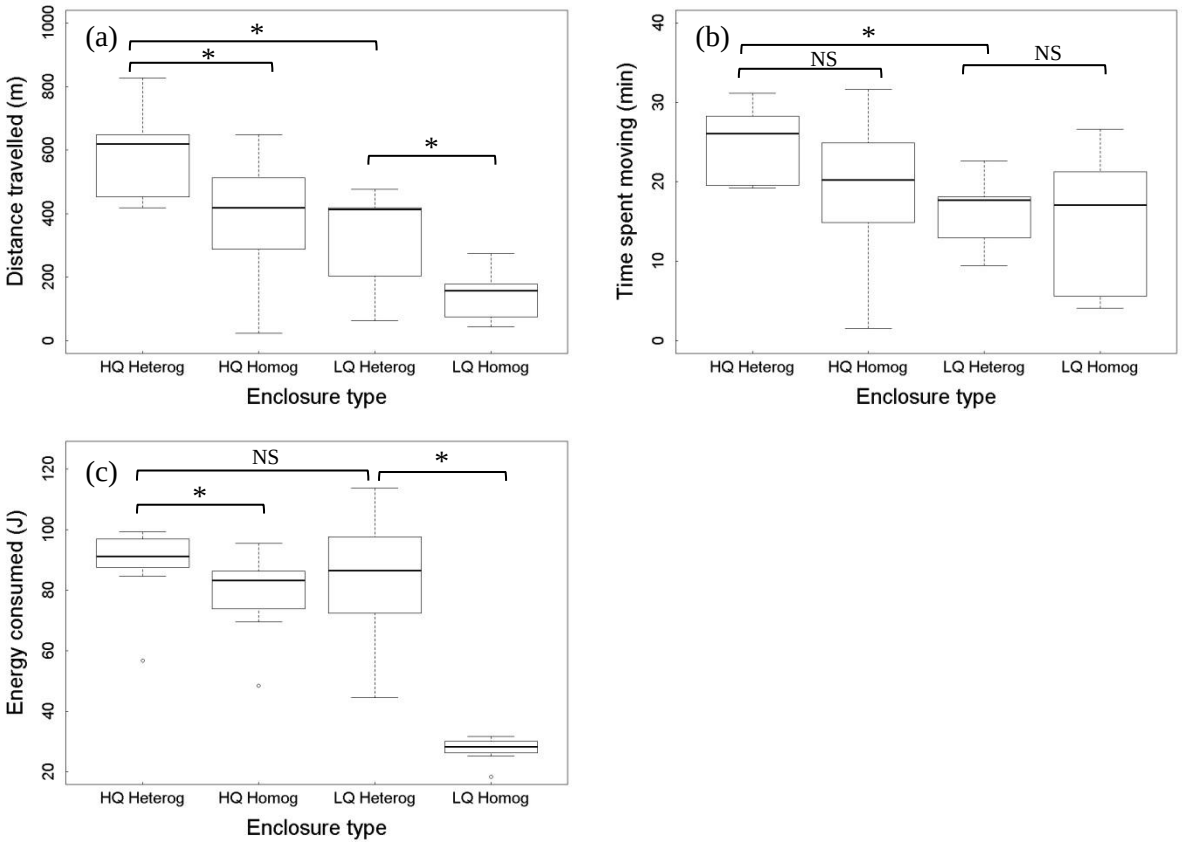

Figure 2.5. Lizard activity in the thermoregulatory enclosures: (a) total distance travelled (m), (b) time spent moving (min) and (c) total energy consumed (J) by the lizards (n = 7). Bars and stars indicate significant differences between enclosure types based on general mixed effects model outputs taking into account non-independence of individuals (see results).

There were no significant linear relationships between $\overline{d}_b$, distance travelled, time travelled, time basking or and mean body mass within any of the enclosure types (Fig. 2.6a,b,c,d). In LQ heterog, larger lizards spent less time resting (away from lights) and more time basking than smaller lizards, although the latter relationship was not significant (r^2 = 0.59, p<0.05, r^2 = 0.50, p=0.08, respectively Fig. 2.6e). Energy consumption was positively related to body mass in each enclosure type (LQ homog: r^2 = 1.00, p<0.001; LQ heterog: r^2 = 0.86, p<0.001; HQ homog: r^2 = 1.0, p<0.001; HQ heterog: r^2 = 0.97, p<0.001, Fig. 2.6f). Despite the few significant linear fits, the relationships between body size and response variables differed across enclosure types. The slopes of $\overline{d}_b$ and mass were significantly different between the HQ heterog and LQ heterog enclosures (homogeneity of slopes test, $F_{12,1}$=6.19, p<0.05, Fig. 2.6a). The slopes of time travelled and mass were significantly different between the LQ homog and LQ heterog enclosures ($F_{12,1}$=5.96, p<0.05, Fig. 2.6c). Additionally, the slopes of time basking and mass and time resting away from the light and mass were significantly different between the LQ heterog and HQ heterog enclosures ($F_{12,1}$=6.27, p<0.05 and $F_{12,1}$=7.09, p<0.05 for time basking and time resting, respectively, Fig. 2.6d and e). Finally, the slopes of energy

consumed and average mass significantly different between the LQ heterog and LQ homog enclosures ($F_{12,1}$=17.83, p<0.005, Fig. 2.6f).

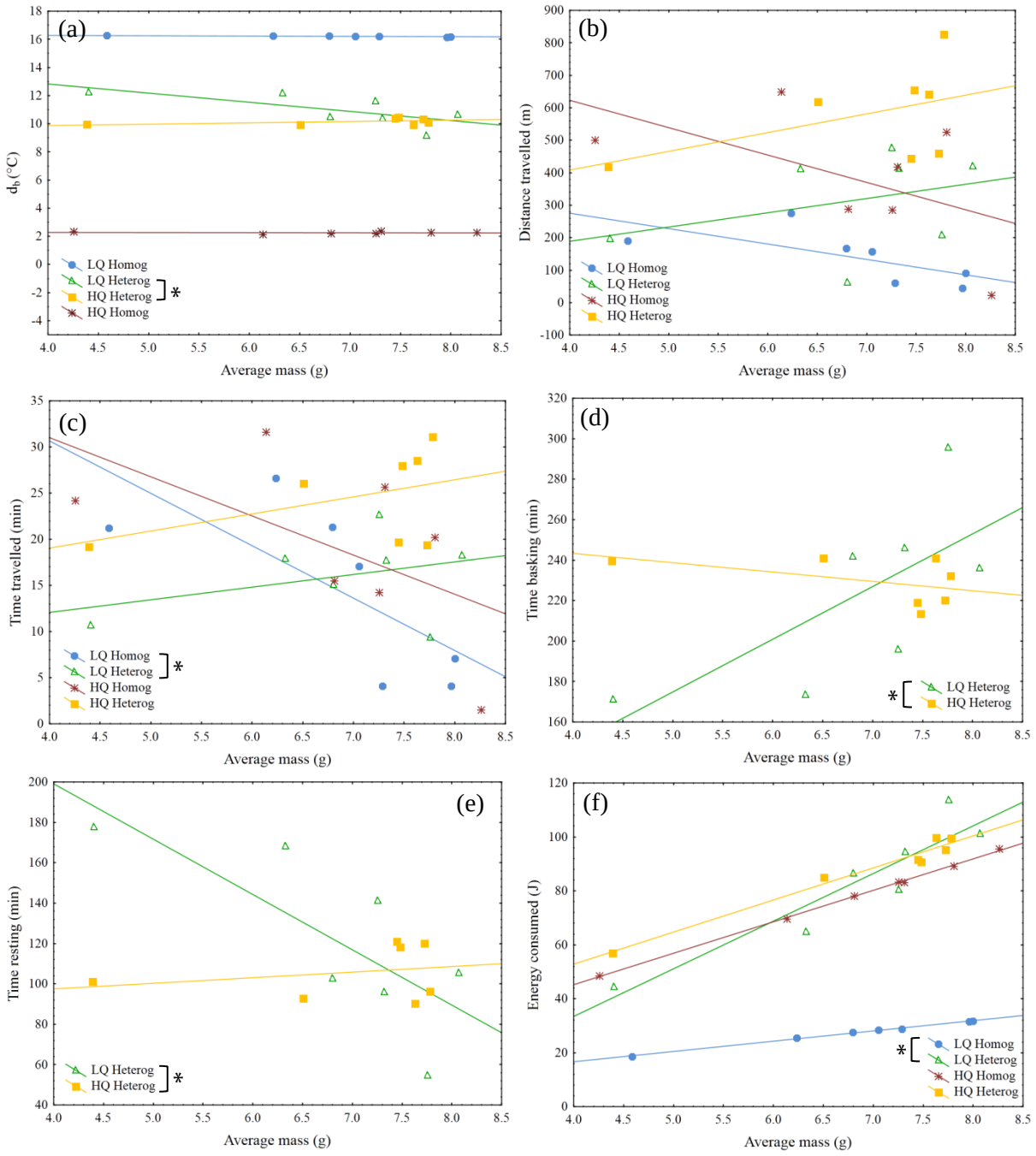

Figure 2.6. Enclosure type and average mass interactions for (a) thermoregulatory accuracy ($\overline{d}_b$) (b) distance travelled (c) time travelled, (d) time basking (lizard under the light) (e) time at rest (not basking) and (f) total energy consumed. Bars and stars in the legends indicate significant differences in slopes between enclosure types (homogeneity of slopes test). Linear regressions found significant relationships between energy consumed in all the enclosure types and average mass and for time resting and average mass in the LQ heterog enclosure. See results for details.

2.4 Discussion

The primary prediction of the CB model states that lizards should invest little into thermoregulation in low quality environments as the costs of doing so are high and likely surpass the benefits. However, the model presents several challenges, in particular, the cost and benefits of thermoregulation are varied and difficult to measure and defining the threshold where costs become higher than benefits is extremely complex. Here, I designed a unique experiment by incorporating four scenarios that only differ in the quality of the thermal environment (including two 'control' scenarios with no possibility of thermoregulation) and relate costs to direct energy expenditure in each enclosure. By using an energy budget approach, I showed that in scenarios where the quality of the environment is low but the opportunity to thermoregulate still exists, the energetic costs of locomotion to thermoregulate are minimal due to the temperature dependence of locomotion and because temperatures away from optimal microsites are generally low. This study therefore provides an explanation for scenarios with limited support for the CB model.

In both LQ homog and LQ heterog, the thermal quality was low (high $\overline{d}_e$) and not significantly different but in the LQ heterog, lizards had the possibility to use a single basking site to raise their temperature (Table 2.1, Fig. 2.4a). Indeed, lizards thermoregulated in the latter enclosure, reflected by a higher thermal accuracy (lower $\overline{d}_b$, Table 2.1, Fig. 2.4b) despite associated costs (higher distance travelled and higher energy consumed than in the LQ homog, Table 2.2). In addition, larger lizards in the LQ homog enclosure spent less time travelling than smaller lizards whereas the opposite pattern was found in the LQ heterog enclosure (Fig. 2.6c). In a thermally poor environment, it is likely cost-effective to avoid spending additional time and energy seeking favourable T_es when the thermal quality of the habitat is consistently low. These effects seem particularly relevant for large lizards, perhaps as a larger size requires more investment of energy for maintenance and growth (instead of other activities, i.e. exploratory movements) than small lizards (Stevenson 1985; Angilletta 2001; Gillooly *et al*. 2001) and thermal inertia may counteract thermoregulatory efforts. Overall, my experimental results for time and energy consumed in the LQ heterog scenario suggest that the conditions were not sufficiently suboptimal to have elicited costs that outweighed the benefits.

Both thermally high-quality enclosures had a high frequency of favourable T_es, with higher quality (lower $\overline{d}_e$) in the HQ homog enclosure since T_e was temporally and spatially maintained at ~30°C (Table 2.1, Fig. 2.3a,b). The HQ homog resembles that of a tropical habitat, in which a random walk maintains body temperature at preferred levels, hence the low $\overline{d}_b$. There is therefore no need to thermoregulate and other routine activities (exploration, foraging, mating) can take place readily. By contrast, lizards in the HQ heterog thermoregulated ($\overline{d}_b < \overline{d}_e$) and travelled greater distances than lizards in the HQ homog, having to exploit heat sources that were spatially and temporally variable (Fig. 2.5a). Despite evidence that lizards in HQ heterog scenarios spent overall more energy, partitioning of the energy budget into locomotion, resting and basking (Table 2.3) showed that

locomotion was not the highest contributor to the costs of thermoregulating in this enclosure. In fact, movements inside the HQ heterog enclosure resulted in lower energetic costs compared to the LQ heterog because lizards were exposed to colder temperatures during movements between heat sources (lights). The higher energy expense was due to the selection of warmer microsites (resting and basking) in the HQ heterog compared to HQ homog (Table 2.4). Despite these thermoregulatory efforts, lizards in the HQ heterog maintained a lower thermal accuracy (higher $\overline{d}_b$, Fig. 2.4b) than those in the HQ homog suggesting that even in a high thermal quality habitat, the heterogeneous nature of the T_e distribution (in space and time) severely reduces lizards' ability to maintain T_b close to T_{sel}.

According to the CB model, lizards in the HQ heterog enclosure should invest more in thermoregulation because the costs are lower than in the LQ heterog. Despite travelling more in the HQ heterog scenario (Fig. 2.5a), lizards did not spend more time basking (Fig. 2.5b) and had similar thermal accuracy as compared with the lizards in the LQ heterog enclosure (Table 2.1, Fig. 2.5c). Therefore, the higher amount of travelling in the HQ heterog enclosure was not associated with thermoregulation as distances between suitable heat sources (lights) were on average shorter than those in the LQ heterog enclosure. By contrast, lizards were more efficient thermoregulators in the poor quality environment (Table 2.1, $\overline{d}_b$ changed by a larger degree relative to $\overline{d}_e$ for lizards in the LQ heterog lizards than in the HQ heterog) despite the higher costs of thermoregulating (moving longer distances between lights). The overall energy consumption was similar between the enclosures (Fig. 2.5c), despite lizards in the HQ heterog enclosure being more active than the lizards in the LQ heterog enclosure (Fig. 2.5b). Therefore, the majority of energy expenditure originated from lizards maintaining high temperatures while basking (see Table 2.3) and not from energy used during locomotion. Overall, lizards in the low cost environment invested more into thermoregulation by efficiently using optimal microsites while lizards in the high quality environment traded off higher accuracy and efficiency for other activities (additional movements, exploration), which is counter to the primary prediction of the CB model. These results are however in line with several other studies that found higher levels of active thermoregulation in more challenging habitats (Grant & Dunham 1988; Blouin-Demers & Weatherhead 2001; Gvoždík 2002; Herczeg *et al*. 2003; Blouin-Demers & Nadeau 2005). Also, by investing more time into thermoregulation, the costs in the LQ heterog are not necessarily energetic, but are likely better represented by missed opportunities (less time to move and other activities).

In addition, larger lizards in the LQ heterog enclosure spent less time resting away from heat sources (lights) and more time basking compared to smaller lizards as opposed to weak effects of size in the HQ heterog enclosure (Fig. 2.6e). These results suggest that in a low quality and heterogeneous distribution of favourable T_es, costs are higher for large lizards and thermoregulation becomes a priority. Size effects have rarely been included in models of thermoregulation (e.g Campbell 1985; Blouin-Demers & Weatherhead 2001; Herczeg *et al*. 2003; Ibargüengoytía *et al*. 2010; Brewster *et al*.

2013, but see Blouin-Demers & Nadeau 2005; Cadena & Tattersall 2009) and this study demonstrates that the balance between costs and benefits may differ substantially between large and small lizards even within a species, and should therefore be included in tests of the CB model. This is clearly important since the basic physics of heat and mass transfer is highly sensitive to body size (Gates 1980).

Brewster *et al*. (2013) recently designed a similar experiment in which they aimed to quantify the energetic cost of thermoregulation on growth rates of hatchlings of the eastern collared lizard *Crotaphytus collaris.* These authors argued that the reduction in growth rates in the treatment with a high energetic cost of thermoregulation was due to the lizards diverting energy from growth to locomotion for thermoregulation. Their study did not however directly measure the energetic cost of locomotion or directly accounted for the energy used as a result of maintaining optimal temperatures. Given that energy consumption is temperature-dependent (Angilletta 2009), the partitioning of energy expenditure for different behaviours in these treatments is essential for inferring energetic costs as the cause of maintenance or abandonment of thermoregulation. My study demonstrates that, at least for *Cordylus oelofseni*, the costs of moving between basking sites are minimal, and lizards target similar body temperatures despite differences in quality of the environment. Therefore, in addition to energetic costs associated to thermoregulation which should incorporate distances travelled and body size, future studies also need to include costs that relate to social interactions, predation and/or missed opportunities (foraging, feeding social interactions) in time and space.

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General conclusions

Species' physiological and behavioural capacities and ability to adapt to rapidly changing environmental conditions, such as those predicted with climate change scenarios, will determine their ability to buffer climate variation. Most current models that investigate global lizard response to climate change are conducted over very coarse spatial and temporal scales, but fail to take into account temperature variation at the micro-site level, the plastic nature of traits or the ability of behavioural thermoregulation to buffer climatic variation, which would provide better estimates for predicting extinction risks and distributions of lizards globally.

The results of this thesis highlight distinct contributions of lizard behaviour and physiology to buffer climate variation depending on the time scale investigated. *Cordylus oelofseni* showed plasticity of both behaviour and physiology, with traits responding to short-term acclimation and seasonal acclimatisation. The plasticity (behaviour and metabolic rate) or lack thereof (water-loss) will likely have important consequences for predictive models of this species' response to climate change. For *C. oelofseni*, the major constraint in maximising energy gain is the poor thermal environment during winter and potential high heat-loads during summer. Further work should translate the benefits provided by the plastic responses, and in light of microsite variation throughout the year and across years, into 'fitness' terms. In particular, it would also be beneficial to incorporate the energetic costs of reproduction and food assimilation in such models.

From a behavioural point of view, these lizards respond readily to their thermal environment as demonstrated in Chapter 2, and buffer temperature variation in the habitat despite associated costs. However, this study has also demonstrated that accounting for costs associated directly with thermoregulation such as locomotion costs is not sufficient to test the primary prediction of the cost benefit model of thermoregulation. Instead, in addition to the energetic costs associated with shuttling across basking sites, the costs of missed opportunities (e.g. foraging) should also be incorporated into the energetic budget. Finally, body mass seems to play a crucial role in the response of lizards to changing thermal landscapes but has been overlooked in most tests of the cost-benefit model of thermoregulation and thus, deserves further exploration.

Addendum A

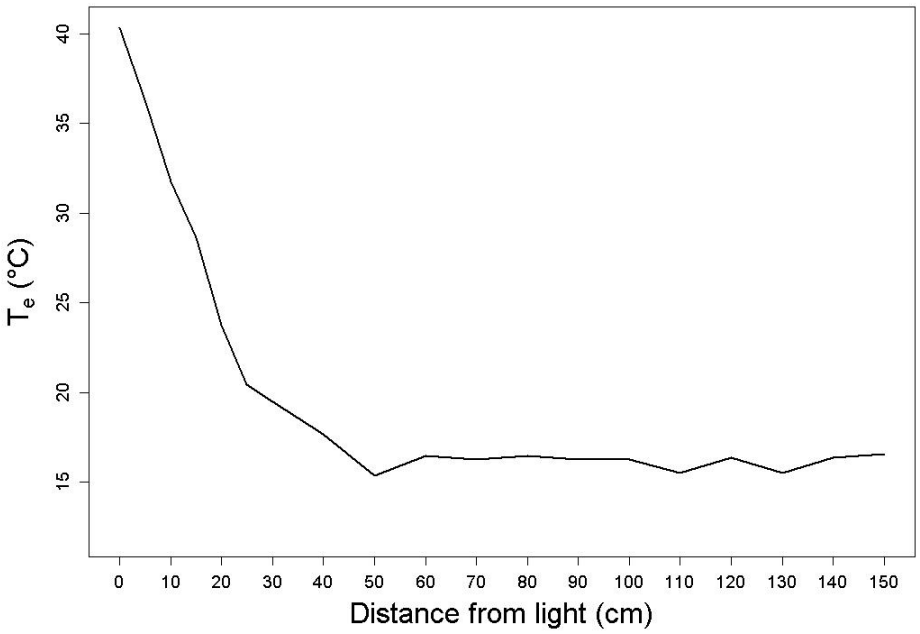

Figure A.1. Frequency distribution profile for operative temperatures, T_e (°C) taken from copper models when a single infra-red light is switched on in an enclosure with the room set at 15°C. Copper models were placed at 0 cm which is directly under the light and up to 150 cm away from the heat source.

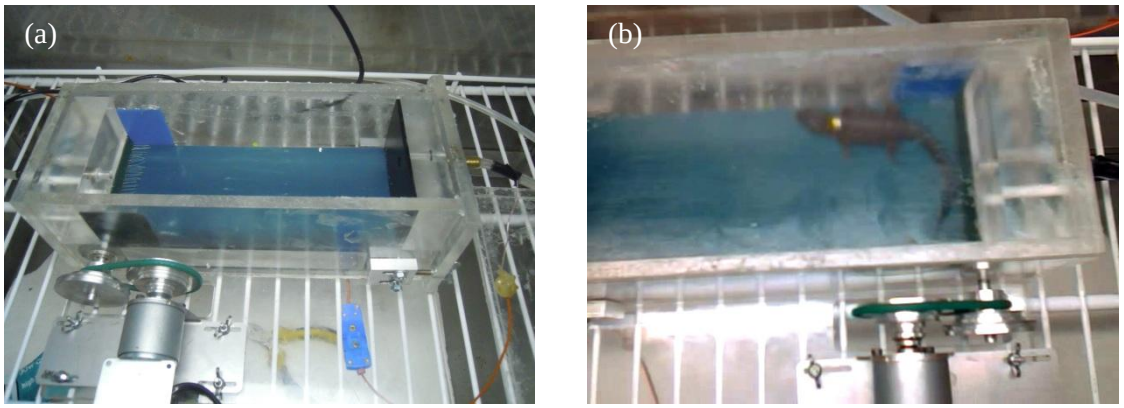

Figure A.2. Customised treadmill used to measure active metabolic rate of lizards. (a) The treadmill was made of Perspex with a rubber band that rotated around two axes by a geared DC motor and placed inside a temperature-controlled incubator (b) Snapshot from a video taken of a lizard running on the treadmill. The treadmill was connected to an infra-red CO_2/H_2O analyser and used to collect active metabolic rate data.

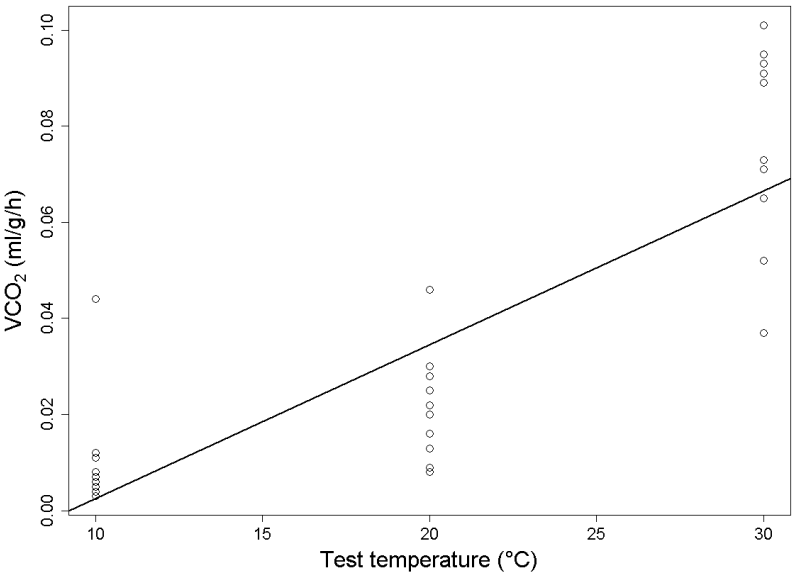

Figure A.3. RMR by test temperature data for male summer- and winter-collected lizards from the 20°C acclimation groups (n = 13, NS differences in RMR between these two groups, t(23)=0.63, p>0.05). The equation of the line describing the significant relationship is RMR = -0.030 + 0.003*Test temperature (r^2 = 0.70, p<0.001).

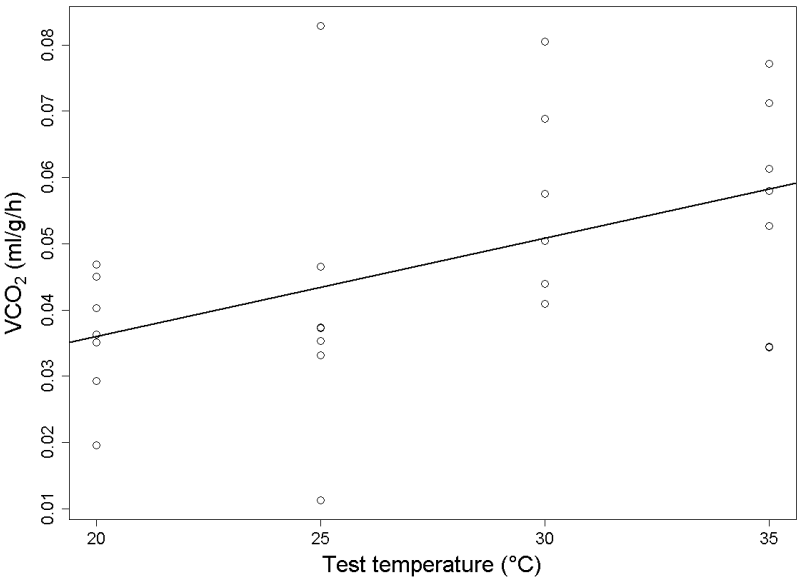

Figure A.4. AMR by test temperature data for male and female summer-collected lizards (n = 7, NS differences in AMR between males and females, t(5)=-2.53, p>0.05). The equation of the line describing this significant relationship is AMR = 0.006 + 0.002*Test temperature (r^2 = 0.023, p<0.05). There were significant differences between summer and winter AMR (t(21)=-3.05, p<0.05), therefore only summer data were used in these analyses.